

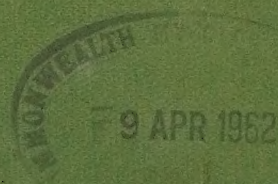
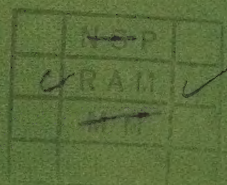
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Respiration of Sugar-Beet Leaves during Illumination, with Simultaneous Photosynthesis

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Dýchání listů cukrovky na světle, probíhající současně s fotosyntézou

Autoři sledovali současně intenzitu dýchání a intenzitu fotosynthesy u listů cukrovky, neoddělených od rostliny. Několik hodin před pokusem asimilovaly listy radioaktivní $^{14}\text{CO}_2$, načež byly umístěny do normální listové komory k průtokovému gazometrickému stanovení intenzity fotosynthesy podle změny koncentrace CO_2 v procházejícím vzduchu. Současně s gazometrickým stanovením fotosynthesy měřili autoři specifickou aktivitu kyslíčnicku uhličitého ve vzduchu, který prošel asimilační komorou. Podle hodnot specifické aktivity CO_2 , kterou vylučuje list v temnotě, je možno vypočítat intenzitu dýchání v mg CO_2 .

Bylo zjištěno, že listy cukrovky vylučují na světle radioaktivní CO_2 , a to jak první tak i druhý den po asimilaci značeného CO_2 . Při silném přehřívání listů v komoře, kdy gazometrickou metodou bylo zjištěno již jen dýchání, radiometricky byl stanoven výdej $^{14}\text{CO}_2$, odpovídající vyšší intenzitě dýchání. Autoři vysvětlují tuto skutečnost tím, že i při přehřívání listů probíhá současně s dýcháním fotosyntetická asimilace kyslíčnicku uhličitého, avšak pasivní bilance CO_2 ve výměně plynů vede ke zjištěnému dýchání, které je v podstatě rozdílem mezi intenzitou plné fotosynthesy a plného dýchání.

Produkce kyslíčnicku uhličitého celými listy cukrovky na světle není za normálních podmínek výživy výjimečným zjevem.

Summary

The intensity of respiration and of photosynthesis were investigated simultaneously in sugar-beet leaves not detached from the plant. Several hours before the experiment the leaves had assimilated radioactive $^{14}\text{CO}_2$ whereafter they were placed into a normal leaf chamber for continuous gasometric estima-

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tion of the intensity of photosynthesis, according to the changes in the concentration of carbon dioxide in the passing air. At the same time, the specific activity of CO_2 was measured in the air that had passed through the assimilation chamber. From the values of the specific activity of CO_2 produced by the leaf in the dark the intensity of respiration in mg. CO_2 can be calculated.

It was found that sugar-beet leaves produce radioactive carbon dioxide when illuminated, both on the first and the second day after assimilation of labelled CO_2 . During the considerable over-heating of leaves in the chamber when the gasometric method did not reveal anything but respiration a high production of $^{14}\text{CO}_2$ could be observed radiometrically, corresponding to a higher respiration intensity. The authors explain this fact by the assumption that photosynthetic assimilation of carbon dioxide takes place simultaneously with respiration but the passive CO_2 balance in the gas exchange results in increased respiration, which actually represents the difference between the intensity of full photosynthesis and that of full respiration.

The production of carbon dioxide by whole sugar-beet leaves under illumination is no exceptional phenomenon under normal nutritional conditions.

Introduction

The question of whether the process of photosynthesis in assimilating organs of plants during illumination is accompanied by respiration has remained unanswered. The relationship between photosynthesis and respiration of green leaves during illumination is not quite clear at present. A number of authors have pointed out the fundamental impossibility of assaying "pure" photosynthesis and respiration during illumination (VOTCHAL 1938).

In calculating the amount of the so-called "pure" photosynthesis the correction for CO_2 production due to respiration was determined from the estimation of the respiration intensity of leaves in the dark. There is no reason, however, to suppose the intensity of respiration in the dark and under illumination to be equal.

The application of radioactive and stable isotopes to simultaneous measurement of the intensity of respiration and photosynthesis during illumination resulted in a number of new findings. A great many of them are rather contradictory so that the problem of the relationship between photosynthesis and respiration in the light has become even more debatable.

Special, carefully planned investigation was carried out with the radioactive isotope ^{14}C by WEIGL, WARRINGTON and CALVIN (1951) and by STEEMAN-NIELSEN (1955), and with the heavy oxygen isotope ^{18}O by BROWN (1953), BROWN and WEIS (1959) and WEIS and BROWN (1959). An interesting view has been advanced by WARBURG (1952), on the basis of his considerations of the quantum yield of photosynthesis.

A part of these studies support the assumption that the light has no significant effect on the respiration of the green parts of plants; on the other hand, the results of numerous experiments carried out by STEEMAN-NIELSEN (1955) and WEIGL et al. (1951) suggest that the production of CO_2 by green

cells is severely blocked by the light and occasionally stops completely.

Thus e.g., BROWN and WEIS (1959) have found in their experiments with the alga *Ankistrodesmus braunii* that, as apposed to what had been claimed before, the light inhibits the process of CO_2 production, the effect being independent of the intensity of illumination. The authors relate this phenomenon to the postulated process of at least a partial replacement of the reducing substances formed in the Krebs cycle or in the pentose-phosphate cycle, by compounds of photosynthetic origin. In other words, that during illumination a sort of blockade of the Krebs cycle takes place which is connected with a decrease in the production of carbon dioxide formed during decarboxylation. This aspect of the relationship between photosynthesis and respiration can be investigated by simultaneously measuring the intensity of the exchange of oxygen and carbon dioxide.

Theoretical considerations of WARBURG (1952) would indicate that illumination should apparently increase the intensity of respiration.

All the above results have been thoroughly discussed in the papers of RABINOWITCH (1956) and ZALENSKY (1957). ZALENSKY (1957) reached the conclusion that the production of carbon dioxide during illumination can proceed under such conditions as represent "a marginal situation for the physiological activity of the leaf and apparently result in an injury to the assimilation apparatus". Under normal conditions, the exchange of CO_2 can possess either the photosynthetic character (during illumination) or the respiration character (in the dark). The exchange of gases during illumination is then not accompanied by any release of carbon dioxide into the medium.

Most investigations of this type were carried out with unicellular green algae or blue-green algae and with isolated parts of higher plants. Therefore, in order to verify the general validity of the above views, further studies are indicated. It is possible that the above-mentioned contradictory results have their origin in the fact that under the specific conditions of the individual experiments the physiological state of the plants may also be different.

The nutrition of plants is undoubtedly of considerable importance, particularly as concerns their supply with nitrogen, as was shown by the work of WEIS and BROWN (1959) and BIDWELL, KROTKOV and REED (1955) who found the blocking to be reversible in the presence of ammonium nitrate. During the investigation of the relationship between photosynthesis and respiration in multicellular organisms, particularly in higher plants, a certain role can be played by the anatomical-morphological distribution of the tissues and cells with either photosynthesis or respiration predominating. On account of this last-named fact, the results obtained by simultaneous measurement of the exchange of oxygen and carbon dioxide may considerably differ from those obtained in unicellular algae.

Of a special interest are the results obtained by DOMAN (1959) who estimated the changes in the composition and concentration of substances formed in the leaves both during illumination and in the dark in an atmosphere of $^{14}\text{CO}_2$ and participating both in photosynthesis and in respiration. This investigation of the relationship between the two processes on the basis of their chemism is doubtless very promising.

In order to elucidate the relationship between photosynthesis and respir-

ation during illumination, divers approaches, methods and objects must be applied. It is the aim of the present communication to contribute to our understanding of these relationships in higher plants, radioactive $^{14}\text{CO}_2$ analyses and the conventional gasometric method being used simultaneously for estimating the intensity of photosynthesis.

Material and Methods

Leaves of the sugar-beet [*Beta vulgaris*, ssp. *esculenta* (SALIB.) GUERKE, var. *altissima* ROESSIG] undetached from the plant were used for the experiments. For estimating the exchange of CO_2 , the apparatus of BATYUK, RIBALCO and OKANENKO (1958) was employed, which makes it possible to carry out short-time measurements of photosynthesis and respiration.

The plant leaf containing radioactive carbon was placed in an assimilation chamber through which air was passing at a relatively high rate. Changes in the carbon dioxide concentration and total radioactivity were estimated in this air.

For the experiments of 1958, plants which had previously assimilated radioactive carbon dioxide, were used. For this purpose the vegetation vessel containing a mature sugar-beet plant (age 120 days) was placed in a plexiglas chamber of 50 l. capacity. All openings were carefully sealed with plastic clay. By acidifying the solution of sodium bicarbonate with labelled carbon, radioactive carbon dioxide of a total activity of 300 μC was obtained. The chamber containing the plant was then exposed to dispersed sunlight. The exposure lasted for 90 min. During the experiments of 1959, only individual leaves were exposed in the atmosphere of $^{14}\text{CO}_2$ (but not detached from the plant). For this purpose a special devise was constructed: a closed circuit with leaf chambers through which air was circulating rapidly. This made it possible to obtain leaves possessing a high tissue activity at exposures of varying length, the possibility of overheating the leaves being excluded.

After "saturating" the plants or individual leaves with radioactive carbon the plants were left in an open area in order to remove all free $^{14}\text{CO}_2$, not assimilated by the plant, from the leaves.

The simultaneous determination of the intensity of respiration and of photosynthesis during illumination was carried out 1, 2 and 3 hours and 1 day after "saturation".

The leaf was placed in a flat chamber of medium size through which air was passing at a rate of 1 l./min. The air was then analyzed in the above-mentioned apparatus (BATYUK, RIBALCO and OKANENKO 1958). All carbon dioxide from the air passing through the assimilation chamber was trapped in a solution of NaOH in a glass absorber. At the beginning the leaf was placed in an ebonite chamber, i.e. in the dark, and respiration was followed for 30 min. Then it was transferred to a plexiglas chamber of the same shape and the intensity of photosynthesis determined. At the same time, the temperature of the chamber and the intensity of insolation was measured. After this, the leaf was again placed in the dark chamber in order to determine the changes in the specific activity of $^{14}\text{CO}_2$ during the experiment. The experiment consisted thus of three parts; the first and third were carried out in the dark and the second under illumination.

The activity was determined in the precipitate of barium carbonate obtained on precipitation of the NaOH solution from the absorber. The standard error of the estimations was about 5%.

According to the changes in the concentration of carbon dioxide in the air the intensity of photosynthesis and of respiration could be assessed. It is correct, however, to speak rather about an apparent intensity of photosynthesis. The appearance of radioactive CO_2 in the air coming from the chamber containing the experimental leaf indicates that CO_2 was given off by the leaf. The total activity of carbon does not permit, however, the obtaining of a complete picture of the amount of carbon dioxide produced by the leaf, since not all CO_2 , particularly that in the intercellular spaces, can be washed out by the air stream. In spite of this, however, the values of overall carbon dioxide activity can serve as a relative indicator of the production of carbon dioxide by the leaf under illumination. These relative values can be mutually compared if the rate of flow of air through the chamber is maintained constant.

In order to be able to estimate at least approximately the amount of carbon dioxide produced during illumination it is possible to employ the relative activity of CO_2 produced in the dark as a basis. Such a calculation is then based on the assumption that the ratio between the radioactive and stable carbon isotopes produced in the form of carbon dioxide during illumination and in the dark is the same.

The intensity of respiration during illumination was derived from the values of the average specific activity of CO_2 respiration in the dark, obtained as the mean of estimations during both dark phases of the experiment, and from the total activity of carbon dioxide produced by the leaf during illumination. The respiration intensity under illumination can be computed from the following formula:

$$I_{\text{light}} = \frac{A}{Y_{\text{dark}}},$$

where A denotes the total activity of carbon dioxide trapped by the NaOH solution in the absorber during the light phase of the experiment, and Y_{dark} denotes the specific activity of carbon dioxide produced by the leaf in the dark.

It was considered necessary to call the attention to these assumptions so as to emphasize that the results of the experiments are only relative. In spite of this, however, the data shed some light on the fundamental principle of the problem: whether carbon dioxide is produced by the leaf even during illumination when the intensity of photosynthesis is high.



Fig. 1. Apparatus for obtaining radioactive plant leaves.

Results

The results of the experiments of 1958 are shown in Figs. 2 and 3 and in Table 1. The intensity of apparent photosynthesis was determined gasometrically, the intensity of respiration under illumination was estimated by measuring the activity of carbon dioxide produced under illumination, and the intensity of full photosynthesis was arrived at as the difference between the value of apparent photosynthesis and respiration under illumination. The plus sign denotes uptake and the minus sign production of CO_2 .

Table 1. Intensity of respiration and of photosynthesis of sugar-beet leaves during illumination and in the dark (1958)

Phase of experiment	mg. CO ₂ /dm. ² /hr.		
	Intensity of observed photosynthesis and respiration	Intensity of respiration during illumination	Intensity of full photosynthesis
Initial dark Light Final dark	2 hr. after assimilation of labelled CO ₂		
	— 4.0	—	—
	+ 7.83	— 0.81	+ 8.64
Initial dark Light	24 hr. after assimilation of labelled CO ₂		
	— 2.9	—	—
	+ 8.0	— 1.61	+ 9.61

The results presented indicate that the leaf produced carbon dioxide during illumination, both on the first and on the second day of the experiment. The first-day intensity of CO₂ production was lower during illumination than in the dark, which suggests that the respiration was partly inhibited by illumination. On the second day, the intensity of respiration under illumination differed from that in the dark considerably less. As follows from the figures, during illumination only photosynthesis could be demonstrated gasometrically.

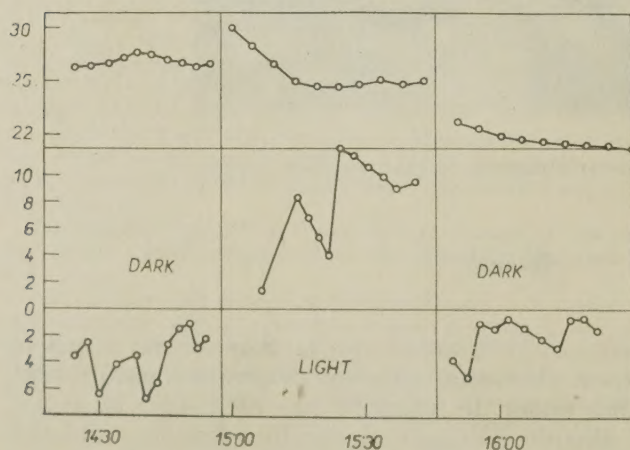


Fig. 2. The changes in respiration and photosynthesis in sugar-beet (August 13, 1958). Abscissa: hours of the day, ordinate: intensity of photosynthesis and respiration in mg. CO₂/dm.²/hr. and the temperature in the chamber in °C.

The results of the experiments of 1959 support the above-mentioned conclusions. Table 2 shows the intensity of respiration (under illumination and

Fig. 3. Changes in the respiration and photosynthesis in sugar-beet (August 15, 1958), two days after assimilation of labelled carbon dioxide. Abscissa: hours of the day, ordinate: intensity of photosynthesis and respiration in mg. $\text{CO}_2/\text{dm}^2/\text{hr.}$ and the temperature of the air in the chamber in $^{\circ}\text{C}$.

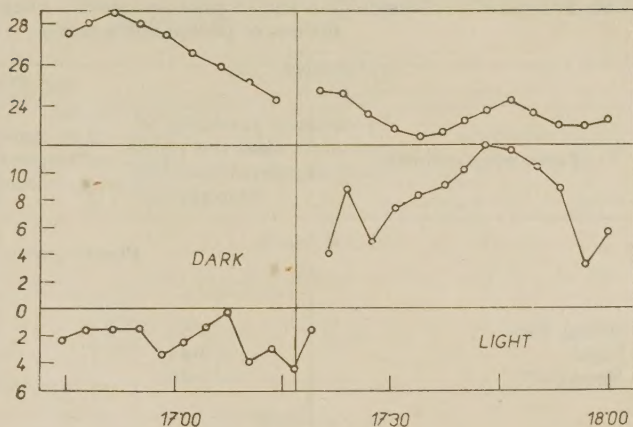


Fig. 4. Changes in the respiration and photosynthesis in the sugar-beet (August 13, 1959).
 ● --- ● : complete nutrition:
 ○ --- ○ : phosphorus deficiency.

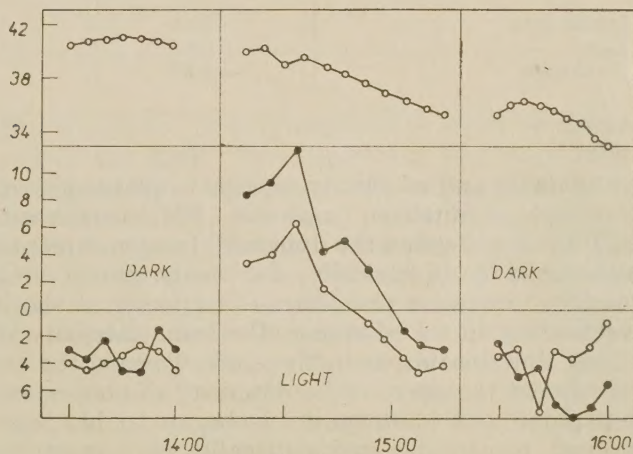


Fig. 5. Changes in the respiration of sugar-beet leaves after over-heating in the assimilation chamber (August 14, 1959). Full line: plant No. 13; broken line: plant No. 5.

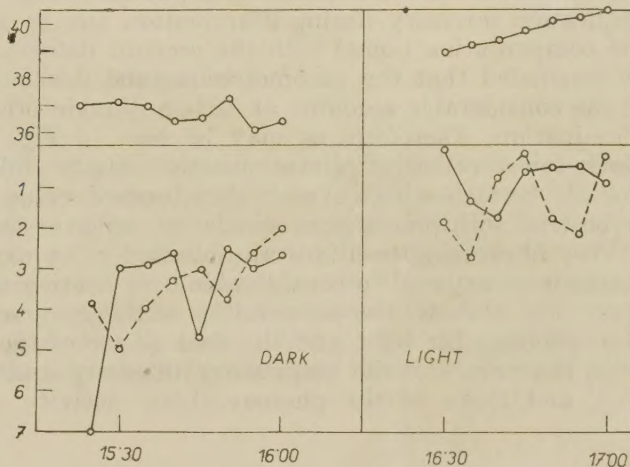


Table 2. Intensity of respiration and of photosynthesis of sugar-beet leaves under various conditions of phosphate nutrition (1959)

Phase of experiment	mg. CO ₂ /dm. ² /hr.		
	Intensity of observed photosynthesis and respiration	Intensity of respiration during illumination	Intensity of full photosynthesis
Plants grown on full nutrition			
Initial dark	— 2.83	—	—
Light	+ 3.43	— 3.24	+ 6.67
Final dark	— 5.40	—	—
Plants grown under phosphate deficiency			
Initial dark	— 3.14	—	—
Light	+ 0.09	— 3.01	+ 3.10
Final dark	— 3.67	—	—

in the dark) and of photosynthesis in plants grown under different conditions of phosphate nutrition (control — PK, variant with phosphorus deficiency — 1/3 PK). Fig. 4 shows the dynamic changes of respiration and of photosynthesis determined gasometrically. In plants grown under conditions of complete nutrition or under phosphorus deficiency, if the leaves were not injured by overheating in the chamber, the same intensity of respiration was observed during illumination as in the dark. Toward the end of the light phase of the experiment the curve of the intensity of photosynthesis reached its compensation point and photosynthesis began to be depressed: carbon dioxide was evolved, measurable gasometrically. On comparing the amount of CO₂ liberated from the leaf and determined gasometrically (bounded by the curve of respiration intensity during illumination and by the straight-line indicating the compensation point) with the amount determined radiometrically it can be concluded that the gasometric method does not permit the determination of the considerable amounts of carbon dioxide produced by the leaves under illumination. Therefore, as may be seen in Fig. 4, the gasometric method yields lower values of photosynthetic activity: full photosynthesis can occasionally be rather high even if the observed value is minute. This was found to be true with plants grown under phosphorus deficiency.

Very interesting results were obtained in an experiment where sugar-beet plants were exposed to considerable over-heating in the course of the experiment. Fig. 5 shows the peculiarities of the gas exchange of the leaves of two plants during the light and the dark phase of the experiment. Table 3 contains the values of the respiratory intensity under illumination and in the dark and those of the photosynthetic activity of these plants. It follow

Table 3. Intensity of respiration in wilted sugar-beet leaves during illumination and in the dark (1959)

Phase of experiment	mg. CO ₂ /dm. ² /hr.		
	Intensity of observed photo-synthesis and respiration	Intensity of respiration during illumination	Intensity of full photosynthesis
	Plant No. 5		
	— 3.54	—	—
	— 1.64	— 3.65	+ 2.01
	Plant No. 13		
Dark	— 3.73	—	—
	— 1.10	— 2.47	+ 1.37
Light			

from Fig. 5 that in plant No. 5 and No. 13 only respiration could be detected both under illumination and in the dark. The respiration was more intense in the dark. The determination of the respiratory intensity under illumination with the aid of radioactive carbon, however, yields higher values of respiratory intensity under illumination. Thus for example, the plant No. 5 produced 3.65 mg. CO₂/dm.²/hr. (determined radiometrically) but only 1.64 mg. CO₂/dm.²/hr. (determined gasometrically). The difference between the two determinations is very likely to be due to the fact that under illumination the respiration is accompanied by photosynthesis; the overall passive balance results in a detection of carbon dioxide production by the gasometric method, i.e. in a finding of respiration which represents the actual difference between the intensity of photosynthesis and of full respiration.

Discussion

A correlation of the results of gasometric determinations obtained in studying the intensity of the gas exchange with those of a radiometric analysis with the aid of ¹⁴C is doubtless of importance for the treatment of the complex problems of respiration and photosynthesis and their relationship. On account of the fact that the leaves were maintained in the experiments described here under conditions resembling those found in nature, it may be assumed that the production of carbon dioxide under illumination does not represent an exceptional case with the sugar-beet even at very high intensities of photosynthesis.

With regard to the possibility of a complete or a partial block of the Krebs cycle during illumination, as mentioned by WEIGL et al. (1951), WARBURG (1952), BROWN and WEIS (1959), the considerable intensity of respiration observed here to accompany photosynthesis was considered as the result of respiration of the non-green parts of the plant, under the assumption that the block takes place in the chloroplasts which represent about 35% leaf dry weight (WHATLEY, ORDIN and ARNON 1951). On the other hand, the presence of ammonium nitrate in the leaves (which may be easily visualized in view of its presence in the nutrient solution) can result in relieving the blocking effect on the Krebs cycle. The results presented here are in agreement with the data of YASNIKOV (see DUSHECHKIN et al. 1958) who placed-labelled sucrose on leaves and observed the evolution of radioactive carbon dioxide from the leaves during daylight.

KOLESNIKOV (1959), from a study of the possible oxidation-reduction processes taking place during photosynthesis and respiration, concludes that both in the dark and during illumination molecular oxygen and carbon dioxide are absorbed and released. GABRIELSEN (1948), studying photosynthesis at different concentrations of carbon dioxide (from 0.002 volume per cent upwards) found that at the low CO_2 concentrations carbon dioxide is produced under illumination at a rate of 1.40 mg. $\text{CO}_2/\text{dm}^2/\text{hr}$. The same intensity of CO_2 production was observed in the leaves kept in the dark (1.45 mg. $\text{CO}_2/\text{dm}^2/\text{hr}$). GABRIELSEN concludes on the basis of these results that respiration proceeds at the same rate under illumination as in the dark but points out that his conclusions are at variance with some of the above-mentioned papers, according to which the intermediates of respiration are re-utilized by photosynthesis before carbon dioxide is evolved.

For a closer comprehension of the relationship between photosynthesis and respiration of plants it is indispensable to apply various methods under various conditions of the physiological activity of the leaves and in different plants.

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О дыхании листьев сахарной свеклы на свету, происходящем одновременно с фотосинтезом

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Авторы одновременно учитывали интенсивности дыхания и фотосинтеза у неотделенных от растения листьев сахарной свеклы. Для этого растение или отдельные листья в специальной установке за несколько часов до начала опытов ассимилировали $C^{14}O_2$, после чего листья помещали в ассимиляционную камеру прибора для определения интенсивности фотосинтеза по изменению концентраций углекислоты в токе воздуха. Одновременно с определением интенсивности фотосинтеза измеряли удельную активность углекислоты воздуха. По удельной активности CO_2 , выделяющейся из листа в темноте, можно рассчитать интенсивность дыхания в мг CO_2 .

Было установлено, что листья сахарной свеклы как в первый, так и во второй день после усвоения меченой углекислоты на свету выделяли радиоактивную CO_2 . При сильном перегреве листьев в камере, когда данные газометрического учета свидетельствовали о том, что происходило только дыхание, радиометрические измерения привели к получению более высоких значений интенсивности этого процесса. Это объясняется тем, что даже при перегреве листьев одновременно с дыханием происходило фотосинтетическое поглощение углекислоты, однако баланс CO_2 в газообмене привел к наблюдаемому дыханию, которое, по сути, является разностью между интенсивностью полного фотосинтеза и полного дыхания.

Выделение углекислоты целыми листьями сахарной свеклы на свету при нормальных условиях питания не представляет собой исключительного явления.

The Effect of Photoperiodic Régime on Vernalization of Winter Wheat

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Vliv fotoperiodického režimu na jarovisaci ozimé pšenice

Na ozimé pšenici odr. Hodonínská holice byla studována otázka vlivu fotoperiodického režimu na průběh jarovisace.

Polní pokusy ukázaly, že krátký den urychluje vývoj, působí-li v době jarovisace. Krátký den aplikovaný po jarovisaci ve všech případech vývoj prodlužoval. Při umělém osvětlení nízké intensity a v podmínkách jarovisačních teplot probíhá jarovisace většinou rychleji na dlouhém dni. Jarovisace v temnotě se uskutečňuje pouze po přidání glycidů. Předpokládám, že i u zelených rostlin je nahromadění určitého množství ergastického materiálu, zejména glycidů, jednou z podmínek průběhu jarovisace. Tohoto nahromadění může být dosaženo jednou prodloužením osvětlení (v podmínkách optimálních jarovisačních teplot a při umělém osvětlení poměrně nízké intensity), jindy, v polních podmínkách, krátkým dnem vyvolávajícím specifickou, fotoperiodicky kontrolovanou růstovou reakci inhibující růst.

Summary

1 Photoperiodic experiments with the effect of short day during vernalization of winter wheat under field conditions have shown that the short day enhances development if applied during vernalization. Short day applied after vernalization prolonged the development.

2. At low intensity artificial illumination and under optimal vernalization temperatures vernalization proceeds the faster the longer the illumination. On increasing illumination intensity differences between the effect of short and long day are obliterated. Vernalization in the dark takes place only if saccharides are added.

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3. It is assumed that even in green plants the accumulation of a certain amount of ergastic material, particularly saccharides, represents one of the conditions of vernalization. This accumulation may be achieved by longer illumination (under conditions of vernalization temperature and low illumination intensity), i.e. by a direct photosynthetic effect, or else by short day (under field conditions) causing a specific, photoperiodically controlled growth reaction which inhibits growth. When vernalization proceeds in the dark saccharides must be supplied.

Introduction

The problem of the effect of photoperiodic régime on vernalization in green plants has not been solved satisfactorily so far. Papers dealing with it date back to the late twenties. The data obtained have on comparison frequently a rather contradictory character (particularly those carried out with winter cereals). Some authors have reached the conclusion that low vernalization temperatures can be fully substituted by uninterrupted illumination, or at least that a long day or uninterrupted illumination have a positive effect on the rate of development at the beginning of ontogeny (and thus also vernalization). It is in this sense that most papers are to be interpreted. On the other hand, it has been found repeatedly that it is this very short day existing at the beginning of ontogeny of winter cereals which enhances vernalization and can possibly substitute for the effect of low temperature.

These problems were encountered by the present author in his work dealing with ecology and physiology of vernalization of winter wheat. It had to be ascertained in what way the photoperiodic regime influences vernalization under different cultivation conditions. Partial results of experiments taking up the given problem are summarized in the present paper.

Material and Methods

Material. The offspring of a single plant of the winter wheat *Triticum vulgare* var. *multurum* AL., variety Hodonínská holice was used. The length of vernalization is 55 ± 5 days for seeds containing 52—55% water and 30—40 days for green plants kept on a long day at $1-4^{\circ}\text{C}$ (KREKULE 1960).

Methods. A part of the work described was carried out in the year 1958 — 1959 under field conditions by means of the method of photoperiodic experiments. The grains were vernalized under a constant water content (53—55%) for different periods of time at $+2 \pm 1^{\circ}\text{C}$. The vernalized variants were then exposed to the short day (9 hours) under field conditions (they were covered between 4 p.m. and 7 a.m.) The control variants were kept on a natural day (14—15 hours). Sowing was carried out in 1 m. rows 8 cm. apart (50 grains per row). Each variant was represented by 3 rows which were treated as a whole. The covering procedure was begun as soon as the second leaf of plants had appeared. The rate of development was evaluated with regard to the length of the period between sowing and the phenological phase of earing (at least 10% plants reached the given phase) and of the period sowing — full earing (at least 50% plants reached the given phenological phase) or else with regard to the ratio of plants in the vegetative and reproductive phase of development (assessed according to a morphological analysis of the shoot apexes of the plants).

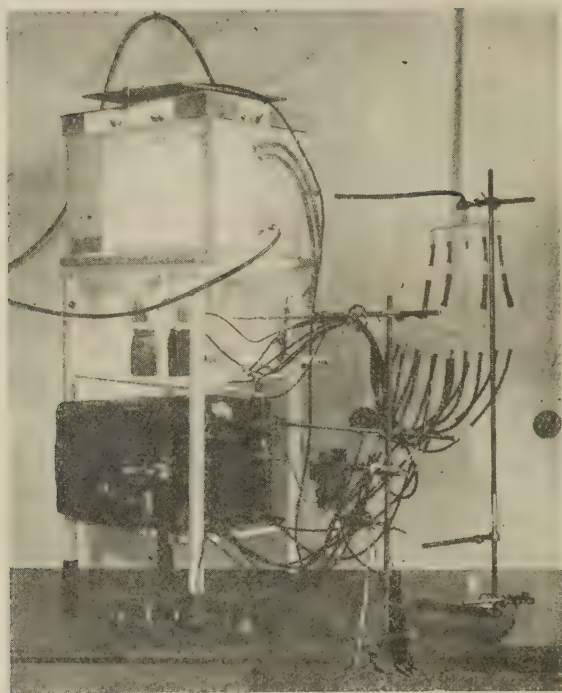
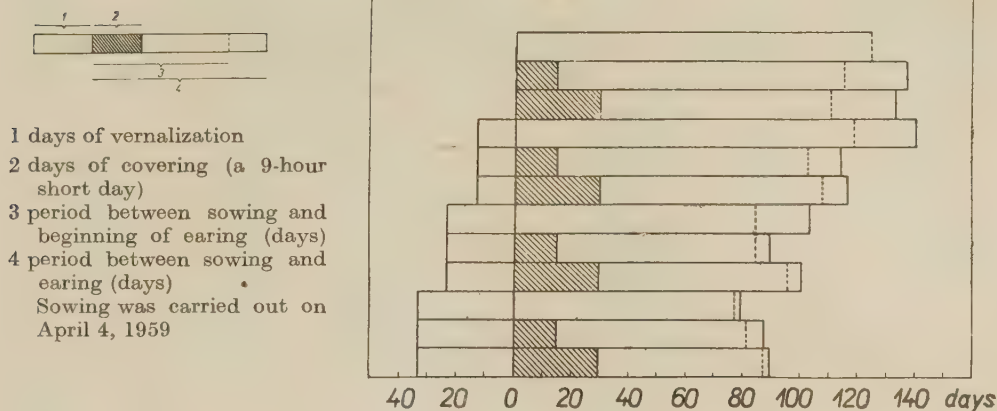


Fig. 1. The experimental arrangement for vernalization of green plants at a higher illumination intensity (the whole apparatus is placed in a cold-room). The dark space which is shown open in the Figure was used for the short-day and darkness variants.

Vernalization at artificial illumination. In 1958, plants were grown under conditions of artificial illumination (a 12-hour photoperiod, 10×25 W white fluorescent tubes illuminating 49 dm^2 from a distance of 75 cm.) at $23 \pm 1^\circ \text{C}$ up to the phase of the second leaf. The plants were then rid of the rest of the seed and vernalized in a cold-room at $2-4^\circ \text{C}$ in the dark, at 8-hour, and at 16-hour illumination period. Four 25 W white fluorescent tubes placed 30 cm. from the cultivation vessels were used for illumination ($1100 \pm 50 \text{ lx}$). During vernalization

the plants were grown as a sand culture in crystallization dishes filled with glass-blower's sand and kept at a constant composition of the nutrient medium. The Knop nutrient solution with the A-Z solution of microelements was used. After termination of vernalization the plants were transferred to flower-pots with garden soil (8-10 plants into one 16 cm. pot) and kept in a greenhouse at temperatures above 15°C . For the evaluation of the experiment the length of the period between sowing and earing has been used as criterion and in plants not taking to ear the degree of differentiation of shoot apices was considered. The scale used has eight grades. For orientation purposes the characteristic of some of them follows: 1 — simple, undivided shoot apex, 2 — shoot apex begins to lengthen, several ridges are apparent at the base, 2.5 — the so-called "double ridge" appears marking the beginning of differentiation of reproductive organs, 3 — spikelet primordia are clearly differentiated, 6 — stamens are differentiated, 8 — the plant is earing. In 1959 the so-called Mischlicht (combined mercury discharge tubes — $6 \times 160 \text{ W}$, Tungsram MBT/U) cooled with a continuous-flow water filter were used for illumination during vernalization. The discharge tubes were placed at a distance of 30 cm. so that an intensity of $8500 \pm 200 \text{ lx}$ was achieved at the level of the cultivation pots. During vernalization the plants were grown as water culture in the Knop nutrient solution (diluted 1:1) containing the A-Z solution of microelements. Reagent flasks of 250 ml. capacity were covered with black, completely opaque paper. Oxygen was passed through these cultivation vessels at a rate of 0.5 l./min. for 9 hours a day. In the variants where the effect of saccharides was investigated vernalization was carried out in the same basic solution with 2% sucrose. The whole arrangement is shown in Fig. 1. The solutions were replaced every 3 to 4 days and the new solution was sterilized before use. Most variants were successfully maintained in a good state under these conditions and only the longest vernalization periods were eliminated on account of bacterial or fungal infection. During illumination the temperature of the lit space rose by $2-3^\circ \text{C}$ and therefore the temperature in the dark had to be raised correspondingly for the same period. Vernalization took then place at the following therm-period: 15 hours $5 \pm 1^\circ \text{C}$, 9 hours $2 \pm 1^\circ \text{C}$. Also in this case plants at the phase of two leaves were used for vernalization, obtained in the same way as in 1958. Further cultivation after vernalization

Fig. 2.



proceeded under the same conditions as during the preceding year. The evaluation of the experiment was carried out after 65 days of cultivation by a morphological analysis of shoot apices (in addition to this experiment, in 1959 the 1958 experiment with vernalization at low intensities was repeated). Non-vernalized plants grown preliminarily at higher temperatures, taking care that their habit will not differ from that of experimental plants after vernalization, were used as controls.

Sugars were estimated by paper chromatography (descending separation in n-butanol-acetic acid-water 4 : 1 : 5). Detection was carried out with 2,3,5-triphenyltetrazolium chloride (a 2% solution in methanol) and subsequent colorimetric estimation after elution with methanol (FISCHER, DÖRFEL 1954). Nonreducing sugars were estimated after hydrolysis with oxalic acid (0.5%, 60 min.) colorimetrically by making use of the Somogyi reaction (NELSON 1944). Sugars were extracted with 80% ethanol.

Results

Fig. 2 and Table 1 show the results of a field photoperiodic experiment. On repeating the experiment at later dates of sowing analogous results were obtained not shown here specifically. It follows from the Figure and from the Table that under the given conditions of the field experiment the short day enhances development (and thus also vernalization) in all cases when it can be assumed that vernalization has not yet taken place. On the other hand, the short day has an inhibitory effect on plants already vernalized. Marked differences may be observed e.g. on comparing the variant without vernalization with that after 33 days of vernalization (Fig. 2). In the first case the short day brought about the earing, in the second, on the contrary, it caused a prolongation of the period up to earing and full earing by some 10 days. In variants with a shorter vernalization period (13 or 23 days) 15 short days caused a greater acceleration than did 30 days. It appears that in this case (30 short days) a part of the short day acted only after termination of vernalization (the plants could finish their vernalization still before appearing above ground).

Table 1

Length of vernalization (in days)	Short day	Date of earing	Length of the period sowing-earing (in days)	Per cent reproductive plants	Number of plants
60	0	29. 6.	58	100	48
60	5	30. 6.	59	100	36
60	10	2. 7.	61	100	42
60	15	3. 7.	62	100	38
60	20	6. 7.	65	100	41
0	0	did not ear	—	24	39
0	5	did not ear	—	24	34
0	10	did not ear	—	28	47
0	15	did not ear	—	40	35
0	20	did not ear	—	51	49

Sowing of all variants took place on May 2, 1959.

Table 2

Length of vernalization (in days)	Photoperiodic régime during vernalization	Date of sowing	Date of earing	Sowing-earing (in days)	Number of plants
35	LD	26. 6.	31. 8.	66	18
35	SD	26. 6.	20. 9.	86	15
35	D	26. 6.	did not ear	—	14
0	—	26. 6.	did not ear	—	18

Legend: LD = 16-hour day — SD = 8-hour day — D = darkness
The experiment was carried out at a low illumination intensity.

Table 3

Variant No.	Length of vernalization (in days)	Illumination and nutrition	Length of the axis in cm. $\bar{x} \pm 3 \cdot s_{\bar{x}}$	Shoot apex phase	Number of plants
1	40	LD	$39.6 \pm 3.3 \cdot 6$	6.8	18
2	40	SD	$39.1 \pm 3.2 \cdot 2$	6.5	20
3	40	SD+S	$38.2 \pm 3.0 \cdot 67$	6.4	22
4	40	D	0	1.1	16
5	40	D+S	$19.3 \pm 3.1 \cdot 4$	3.5	18
6	30	LD	$28.6 \pm 3.1 \cdot 4$	4.8	30
7	30	LD+S	$26.6 \pm 3.1 \cdot 0$	4.6	26
8	30	SD	$28.4 \pm 3.1 \cdot 6$	4.1	22
9	30	SD+S	$27.8 \pm 3.1 \cdot 0$	4.6	20
10	30	D	$1.0 \pm 3.0 \cdot 16$	1.4	22
11	30	D+S	$6.5 \pm 3.0 \cdot 6$	2.1	35
12	control.		$1.1 \pm 3.0 \cdot 24$	1.4	27

Legend: LD = 15-hour day — SD = 9-hour day — D = darkness + S = with 2% sucrose
Control = non-vernalized plants in the same growth phase as variant No. 1.

The experiment was carried out at a high illumination intensity.

The results of experiments where the plants were vernalized at artificial illumination are shown in Tables 2 and 3. In the 1958 experiment (Table 2) the short day had a marked inhibitory influence on the course of vernalization and in the dark the plants were not vernalized at all. Even in the experiment of 1959 at a higher light intensity (Table 3) the long day appears generally more suitable for vernalization than the short one. At 40 days of vernalization, however, the difference between the short-day and the long-day variants is insignificant (it may be assumed that the minimum period of vernalization is shorter for both variants and the differences are obliterated). At 30 days of vernalization the difference is significant. Variants vernalized in the dark remained vegetative in all cases. The effect of sucrose was marked in variants vernalized in the dark for 40 days when the application of sucrose made vernalization possible (shoot apices from variant No. 5 are in the phase of reproductive differentiation already). A lower difference was observed after 30 days of vernalization (variant No. 10 and 11). On a short day at 30 days vernalization the sucrose enhanced the development to a certain degree.

Tables 4 and 5 show an orientation review of some data concerning the growth characteristic of plants in field conditions (Table 4) and during vernalization at artificial illumination (Table 5). The tables also contain data on the content of saccharides in the leaves. An analogous character of the differences between plants from the short and the long day was found to exist even for other dates of sowing (5 in all) and on repeating twice the experiment with vernalization at artificial illumination. The values presented indicate that under field conditions the short day brings about growth inhibition (a characteristic morphological sign of this growth inhibition is shown in Fig. 3) reflected particularly in a shortened leaf blade, sheath and in creeping growth. Tillering is affected but very little. The content of saccharides in leaves, however, increased in consequence to this short-day reaction. Characteristic features of this short-day reaction are to be seen both in vernalized and non-vernalized plants (it is without saying that these features disappear in vernalized plants on shooting). At artificial illumination and under optimal vernalization temperatures no such growth reaction was observed in the experiments described here. Plants from the long and from the short day did not fundamentally differ in this case in their growth characteristic. Sugar content was higher in long-day plants.

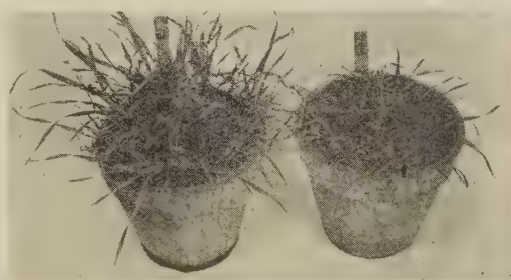


Fig. 3. The growth reaction caused by short day. Left — plants from long day. Right — plants from short day

Table 4

Variant	Number of plants	Tillers $\bar{x} \pm 3 \cdot \bar{s}_x$	Length of leaf-blade of 3rd leaf (mm.) $\bar{x} \pm 3 \cdot \bar{s}_x$	Length of sheath of 3rd leaf (mm.) $\bar{x} \pm 3 \cdot \bar{s}_x$	Length of leaf-blade of 4th leaf (mm.) $\bar{x} \pm 3 \cdot \bar{s}_x$	Fresh weight of 1 plant* (g.) $\bar{x} \pm 3 \cdot \bar{s}_x$	Dry weight of 1 plant (g.)* $\bar{x} \pm 3 \cdot \bar{s}_x$	Per cent dry weight	Sugar content in the leaf-blade of 2nd and 3rd leaf (mg./g. dry wt.)**		
									Fructose	Glucose	Sucrose
Short day	10	$4.1 \pm 3.0 \cdot 35$	$100 \pm 3.4 \cdot 0$	$32 \pm 3.1 \cdot 7$	$87 \pm 3.3 \cdot 8$	$1.08 \pm 3.0 \cdot 079$	$0.220 \pm 3.0 \cdot 012$	19.4	3.66	5.00	8.00
Short day + vernalization	12	$4.8 \pm 3.0 \cdot 35$	$115 \pm 3.4 \cdot 4$	$42 \pm 3.4 \cdot 9$	$109 \pm 3.3 \cdot 7$	$1.51 \pm 3.0 \cdot 083$	$0.295 \pm 3.0 \cdot 018$	19.5	5.72	4.12	7.80
Long day	12	$5.9 \pm 3.0 \cdot 61$	$170 \pm 3.6 \cdot 5$	$60 \pm 3.4 \cdot 7$	$160 \pm 3.8 \cdot 7$	$3.69 \pm 3.0 \cdot 174$	$0.562 \pm 3.0 \cdot 065$	15.2	2.16	1.48	3.60
Long day + vernalization	10	$5.6 \pm 3.0 \cdot 48$	$168 \pm 3.9 \cdot 3$	$62 \pm 3.3 \cdot 5$	$163 \pm 3.11 \cdot 5$	$2.23 \pm 3.0 \cdot 245$	$0.409 \pm 3.0 \cdot 054$	18.3	1.74	1.28	3.20

*) without roots — **) traces of raffinose found in all samples

The plants were evaluated 35 day after sowing (July-August). Differences between the short-day and long-day variants are significant with the exception of the number of tillers. The sugar content was not treated statistically.

Discussion

If the results of the above experiments are summarized the following contradiction appears: Under field conditions the short day enhanced vernalization, at artificial illumination the opposite was true or else there were no significant differences between the two photoperiodic régimes.

A similar effect of the photoperiod is mentioned in the paper of RAZUMOV (1957). This contradiction, depending on experimental conditions, may be observed on comparing the literature pertaining to the subject. Under field conditions, a positive effect of the short day on winter cereals was found e.g. by MAKSIMOV and POYARKOVA (1924), LEBEDINTSEVA (1933), OLENIKOVA (1933), PURVIS (1934), MCKINNEY and SANDO (1933, 1935), BELL (1936), PURVIS and GREGORY (1937), VOSS (1938), TAMM and PREISLER (1938), RAZUMOV et al. (1954), GOTT et al. (1955), YORDANOV (1957), FYODOROV (1959). KRIZHILIN et al. (1959). PETERSON and LOOMIS (1949) found the similar effect in *Poa pratensis* NEWELL in *Bromus inermis* (1951), WYCHERLEY (1952) and GARDNER and LOOMIS (1953) in *Dactylis glomerata* COOPER (1960), EVANS (1960) and FEJER (1960) in some *Lolium* species. Razumov's ecological school explains the effect of short day during vernalization by an influence on the rate of growth, PURVIS and GREGORY (1952) proceed from their developmental scheme to reach the conclusion that the formation of precursors of florigen is faster on a short-day régime. JUNGES (1957) and similarly

Table 5

Variant	Number of plant	Length of leaf-blade of 1st leaf $\bar{x} \pm 3 \cdot s_{\bar{x}}$	Fresh weight of 1 plant (mg.) ¹ $\bar{x} \pm 3 \cdot s_{\bar{x}}$	Dry weight of 1 plant (mg.) ² $\bar{x} \pm 3 \cdot s_{\bar{x}}$	Per cent dry weight	Sugar content in the leaf-blade of the 1st and 2nd leaf (mg./g. dry wt.)**		
						Fructose	Glucose	Sucrose
Short day	10	10.5 ± 3.0	92.1 ± 3.2	14.3 ± 3.0	15.5	7.92	8.10	16.8
Long day	10	11.2 ± 3.0	91.1 ± 3.6	16.4 ± 3.0	17.9	10.24	14.50	28.0

* without roots

** traces of raffinose found in both variants

The plants were vernalized for 20 days in a cold-room (for cultivation conditions see the text) at a high illumination intensity.

Differences between the two variants are insignificant (sugar content was not treated statistically).

FYODOROV (1959) consider generally the short-day requirement during vernalization of winter cereals as one of the features of adaptation to the historical conditions of cultivation (under natural conditions vernalization takes place in autumn under short-day conditions). During vernalization under field or greenhouse conditions a positive effect of the long day on the rate of development and thus on vernalization (in comparison with the short day) was established, or else the short day had no developmental effect, e.g. in the following papers: DENFFER (1939), OVECHKIN (1957), LISTOWSKI (1958), LISTOWSKI and JESMIANOWICZ (1959) and PAVLOV (1958). In the work of HÄNSEL (1951) short day was always inhibitory, when applied after vernalization. A positive effect of the long day during vernalization at low temperature only and at artificial illumination is maintained by RAZUMOV (1957), DOLGUSHIN (1958) and MARKOWSKI (1959). A number of authors most recently also GOTT et al. (1955) obtained experimental material indicating that uninterrupted illumination is optimal for the ontogeny of winter cereals.

The interpretation of all these contradictory results is complicated by the fact that no reliable indicators are at our disposal for the course of vernalization and that we thus cannot determine exactly enough whether a selected photoperiodic régime affects solely the vernalization process or also a further period of the photoperiodic flower induction itself.

In interpreting our results obtained under field conditions and at artificial light and low temperatures it must be borne in mind that the results were obtained not only under a different light régime but also at different temperatures and that they cannot be mutually compared. We are interested here in the contradictory effect of a given photoperiodic régime under the two above types of conditions of cultivation.

The short day, as compared with the long-day period accelerated the development (vernalization) under field conditions and inhibited at artificial illumination. In the first case the short day caused a characteristic limitation of growth and thus a relative increase in dry weight and sugar level. In the second case the short day did not bring about any morphologically marked growth reaction and the dry weight and sugar level decreased.

The importance of the ergastic reserve substances for vernalization was convincingly demonstrated in winter wheat by KRUSHILIN and SHVETSKAYA (1960). The positive effect of short day (under field conditions) as being due to an influence on growth is described by RAZUMOV et al. (1954), OLEINIKOVA (1957), YORDANOV (1957) and RAZUMOV (1957). The mechanism itself and other ecological conditions of this short-day effect have not been explained so far. Some data (a morphological characteristic) about the short-day reaction of growth are presented for

a number of species by LISTOWSKI and JESMIANOWICZ (1960). At artificial illumination (when growth was blocked by low temperature) the effect of light on vernalization is to a considerable extent proportional to its photosynthetic effect and the long day is then more effective. These data are in agreement with the results of authors who investigated the effect of the photoperiod on vernalization under optimal vernalization temperatures and artificial illumination (in this connection the factor of light quality is neglected although it may be more effective in this respect than photoperiodicity itself — PAVLOV 1958).

The possibility that in the experiments described here we may be dealing first of all with an accumulation of ergastic reserve substances, particularly of saccharides, as one of the important conditions of vernalization, is supported by the finding that at a higher illumination intensity the differences between the short and the long day were decreased. On the other hand, at low light intensities there was a considerable difference between the short-day and the long-day variant. The results of an experiment not described in detail here should be mentioned here where the rate of vernalization (at low light intensities) depended on the leaf area developed before vernalization. The possibility itself of vernalization of green plants in the dark after supplying sugars was demonstrated by TASHIMA (1953) in *Raphanus sativus*; the positive effect of sucrose is also mentioned by RAZUMOV et al. (1954). There exist a number of papers describing a similar effect in biennial plants. On the contrary, in the 49-day-old *Arabidopsis thaliana* found NAPP -ZINN (1960) no effect of light on vernalization (in comparison with dark).

It follows from the above-said that if the photoperiodic requirement of green plants during vernalization is to be characterized utmost attention must be devoted first of all to the growth effect dependent not only on the corresponding photoperiod but also on other climatic conditions.

It remains completely unclear, however, to what extent a given photoperiodic régime can have an autonomous effect rather than act via vernalization. In the experiment with short day described here under field conditions vernalization appears to be affected by the short day directly. On the other hand, as shown by some late experiments of FYODOROV (1960) a light of high intensity (uninterrupted illumination) can replace low temperature even in cereals with a pronounced vernalization requirement. On the other hand, WELLENSTIEK (1953) succeeded in *Campanula medium* to replace low temperature by short day, the plant possessing an obligatory vernalization requirement. The author is of the opinion that we are dealing here with a vernalization-independent mechanism. Also MATHON (1959) observed that short days could replace low temperatures in *Symphycandra hoffmani*. Similarly CHOUARD (1957) working with *Scabiosa succisa* and COOPER (1960) working with some *Lolium* species assume an independent induction effect of the short days. These problems cannot be solved within the scope of this paper but the short day alone could not fully replace the effect of low temperature in the experiments not described here in detail.

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Влияние фотопериодического режима на яровизацию зеленых растений

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На озимой пшенице сорта Годонинская голица изучался вопрос влияния фотопериодического режима на течение яровизации. В наших исследованиях использован метод полевых фотопериодических опытов и яровизация в разных фотопериодических режимах при искусственном освещении.

Полевые опыты показали, что короткий день ускоряет развитие, если влияет во время яровизации. Короткий день, примененный после яровизации во всех случаях тормозил развитие. При искусственном освещении низкой интенсивности и в условиях яровизационных температур яровизация протекает тем быстрее, чем длиннее освещение. Яровизация зеленых растений в темноте происходит только в случае добавления глицидов. Полагаю, что свет действует в течение яровизации прежде всего посредством фотосинтеза и не только типичной фотопериодической реакцией. Предполагаю, что и у зеленых растений накопление глицидов является одним из условий течения яровизации. Этого накопления возможно достичь в одном случае изменением освещения (в условиях яровизационных температур и сравнительно низкой интенсивности освещения), в другом случае, в полевых условиях, коротким днем, который вызывает специфическую, фотопериодически регулируемую ростовую реакцию.

Antagonism Between *Rhizoctonia solani* KÜHN and Certain Soil Saprophytes: A Laboratory study

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Antagonismus mezi *Rhizoctonia solani* KÜHN a určitými půdními saprofity.
Laboratorní studium

U tří saprofytů byl sledován projev antagonismu proti pathogenu *Rhizoctonia solani* v různých podmínkách kultivace. *Trichoderma viride* a *Chaetomium cochlioides* inhibovaly růst *R. solani* účinněji při inkubační teplotě 25 a 30° C. *T. viride* bylo antagonisticky aktivnější v prostředí kyselém, *C. cochlioides* v prostředí alkalickém. Je vysloven předpoklad, že půdní doplňky zvyšující aktivitu *C. cochlioides* by mohly být platným opatřením proti sledovanému pathogenu v alkalických půdách. Na rozdíl od předešlých zpráv byla kultura *R. solani* celulólyticky zaktivnější než *T. viride* a *C. cochlioides*, a to i při použití chloridu amonného jako droje dusíku.

Summary

Antagonism of three saprophytes to the pathogen, *Rhizoctonia solani* was studied in plate cultures under different conditions of temperature, pH, and nutrition. The antagonists *Trichoderma viride* and *Chaetomium cochlioides* were found more effective in the inhibition of the growth of *R. solani* at temperatures 25 and 20° C and this conforms to their growth-temperature relationship. *T. viride* was found more effective in acid media as reported by others, whereas other antagonists and especially *C. cochlioides* was more effective in the alkaline medium. Hence the suggestion is made that soil amendments increasing the activity of *C. cochlioides* might be a good control measure against the pathogen in alkaline soils.

The most interesting result of the investigation is that, contrary to the previous reports, *R. solani* appeared to be more cellulolytic than *T. viride* and *C. cochlioides* and this result was unaffected by the use of NH_4Cl as N-source.

Introduction

It is now a common knowledge that during the growth of pathogens in pure culture, contamination by other organisms frequently retards or prevents their growth. The discovery of this phenomenon has led to the study of antagonistic effects between organisms and also to the adoption of antibiotic therapy against human and plant diseases.

FAWCET (1931) was the first to emphasise the use of known mixtures of organisms in plant disease investigations and to suggest that the development of many plant diseases may be very much influenced by associated organisms. Thereafter, various workers like WEINDLING (1934), SANFORD (1946) and WOOD (1951) claimed in greenhouse studies that cultures of antagonists or their culture-filtrates added to the soil containing pathogens reduced or suppressed the development of disease. Although the success of the soil amendments or use of antibiotics for seed dressing is being realised, yet the value in understanding empirically the antagonistic phenomenon under different conditions cannot be overlooked. Hence in this paper the antagonism of saprophytic soil organisms, *Trichoderma viride*, *Penicillium clavariaeforme* and *Chaetomium cochlioides* against *R. solani* under different conditions of temperature, pH, and nutrition has been reported.

Material and Methods

Source of culture: *T. viride* and *C. cochlioides* were obtained from stock culture collection of the Imperial College, London and originally isolated from soil. *P. clavariaeforme* was isolated from a contaminated culture of *R. solani*. They were all maintained on potato dextrose agar (PDA) slants. *R. solani* was isolated from a diseased seedling of Swede.

Media: The following media were used:

(1) PDA: 20% peeled potato slices, 2% glucose & 2% agar. pH 5.9.

(2) Glucose, starch, cellulose powder or sodium carboxy-methyl cellulose: 1%, NH_4Cl or NaNO_3 : 0.144% and 0.227% respectively; KH_2PO_4 : 0.1% and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.05% and agar 2%.

In order to prepare media distilled water was used. The initial pH of the glucose- NH_4Cl medium = 5.2; of glucose- NaNO_3 medium = 5.4, starch- NH_4Cl medium = 5.9, starch- NaNO_3 medium = 6.1, cellulose- NH_4Cl medium = 5.3, cellulose- NaNO_3 medium = 5.1, Na-carboxy-methyl cellulose (CMC) — NH_4Cl = 5.2, CMC — NaNO_3 5.3.

An equal quantity (i.e. 15 ml.) of agar media was sterilised in tubes at 15 lbs. pressure for 20 minutes and plated in the sterile petri dishes.

Method of Inoculation: Spore suspension of the antagonists and 4 mm. disc from the 3–4 days old culture of the pathogen growing on PDA were used as inocula in all the tests.

Ringing Method: Three discs of culture of the pathogen were plated on each agar plate. A No. 12 cork-borer (12 mm. diameter) was dipped in spore suspension of the antagonists placed on the agar and then removed to leave a ring of spores around each disc. Observations were made every day to see under what conditions the pathogen could grow through the ring and to study the growth of antagonists. All treatments were in duplicate and the results after 4 days incubation are given below in the tables.

Table 1. Growth (mm./day) of the antagonists and the pathogen at different temperatures

Organism	Temperature °C					
	30	25	20	15	10	5
<i>R. solani</i>	1.5	16.5	13.0	12.6	7.0	4.5
<i>T. viride</i>	9.0	22.5	14.5	9.0	2.0	0.0
<i>P. clavariaeforme</i>	0.0	4.0	3.0	3.0	2.0	0.0
<i>C. cochlioides</i>	1.2	10.0	6.5	5.5	1.5	0.0

Table 2. Antagonism against *R. solani* at different temperatures

Antagonist	Temperature °C				
	25	10	15	10	5
<i>T. viride</i>	+	+	+-	--	--
<i>P. clavariaeforme</i>	—	—	—	—	—
<i>C. cochlioides</i>	++	+-	—+	—	—

++ : The pathogen confined inside the ring in all six colonies.

+: The pathogen penetrated the ring, and grown outside but the antagonist pursued it from behind and over-ran the pathogen in all six.

+-: More than three and less than six colonies attacked by the antagonist.

—+: Less than three colonies attacked by the antagonist.

—: Pathogen affected inside and just outside the ring, but later escaped the attack.

— —: Pathogen unaffected.

Table 3. Growth of the antagonists and the pathogen at different pH values

Organism	pH				
	4.3	4.9	5.4	6.2	7.7
<i>R. solani</i>	14.0	15.3	16.6	18.0	10.3
<i>T. viride</i>	27.3	27.0	28.0	24.6	6.0
<i>P. clavariaeforme</i>	3.6	3.6	3.6	4.6	2.0
<i>C. cochlioides</i>	6.3	7.3	8.6	10.0	8.6

Table 4. Antagonism against *R. solani* at different pH values

Antagonist	pH			
	4.3	5.4	6.6	8.0
<i>T. viride</i>	++	++	+-	—+
<i>P. clavariaeforme</i>	+-	—+	—+	++
<i>C. cochlioides</i>	—+	+-	+	++

Results and Discussion

1. The growth of the pathogen and the antagonists is compared at different temperatures on PDA and results are given in Table 1.

In the preliminary experiments the optimum temperature for the growth of fungi under study was found. It corresponded to the temperature, of 25° C as reported in the Table 1. *T. viride* grows more rapidly at the temperature of 30°, 25° and 20° C. Below this temperature its growth is less than that of the pathogen. This indicates that in regions where the soil temperature is 20° C and above, this soil saprophyte might check the growth of the pathogen but below 20° C the pathogen might escape the attack of the antagonist. Regarding other antagonists their growth is too slow to check the growth of the pathogen at any temperature from 5 to 30° C.

2. Next, antagonism at different temperatures was investigated and the results are expressed in Table 2.

From the above data it is clear that the antagonistic effect of *T. viride* appeared to be related to its growth at different temperatures in pure culture. But this was not the case with other antagonists. *C. cochlioides* was relatively slower in growth in pure culture but effectively checked the growth of the pathogen at 25° C and also at 20° C. This result conforms to its growth-temperature relationship. *P. clavariaeforme* as reported by WOOD (1951) seems to have a doubtful antagonistic effect. In spite of the complete escape of the pathogen from attack by this fungus its spores, after germination, first deformed and then completely destroyed hyphae of the pathogen on both sides of the ring. As the spores of this antagonist are dispersed in the soil by means of various agencies, it may serve as a very effective antagonist against the pathogen.

3. The growth of organisms at different pH values was studied and the results are reported in Table 3.

They indicate that *T. viride* is potentially useful against *R. solani* under acid conditions whereas other antagonists are not, at all pH values.

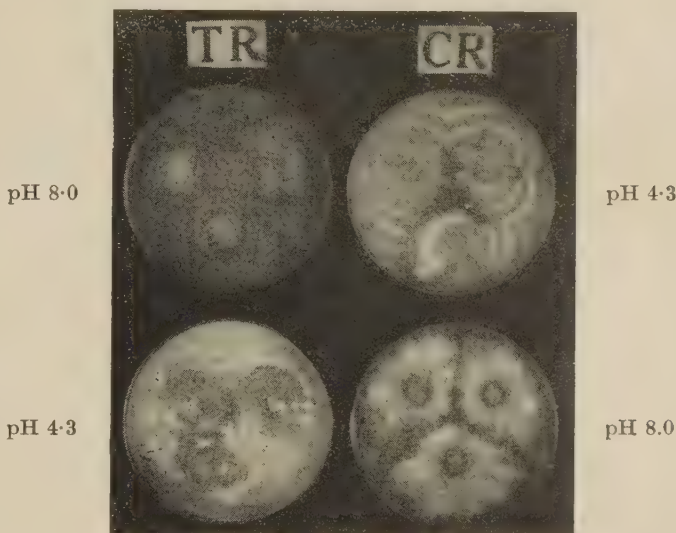


Fig. 1. Antagonism of organisms against *R. solani* at different pH values. — TR = *T. viride* + *R. solani*, CR = *C. cochlioides* + *R. solani*.

4. Antagonism at different pH values was next investigated and the results are presented in Table 4 and Figs. 1 and 2:

The above result supports the view that *T. viride* may be effective in the control of pathogen in acid soils. As regards the antagonistic effect of *P. clavariaeforme* it is rather interesting to note its maximum effect at pH 8.0 and 4.3 and correspondingly less effect at other pH values. This result was quite unexpected in view of its growth in pure culture (Table 2 and Fig. 2). *C. cochlioides* in its effects be-

haves in an opposite way from *T. viride*. Although no previous information is available as regards the pH antagonism relationship of this fungus, it seems probable that it may be more effective in the control of the pathogen in alkaline soils.

5. Growth of organisms in enriched agar media. It has been reported by BLAIR (1941) and WOOD (1951) that addition of wheat straw or maize meal to the soil gave protection to the seedlings against the pathogen. BLAIR explained this as being due to

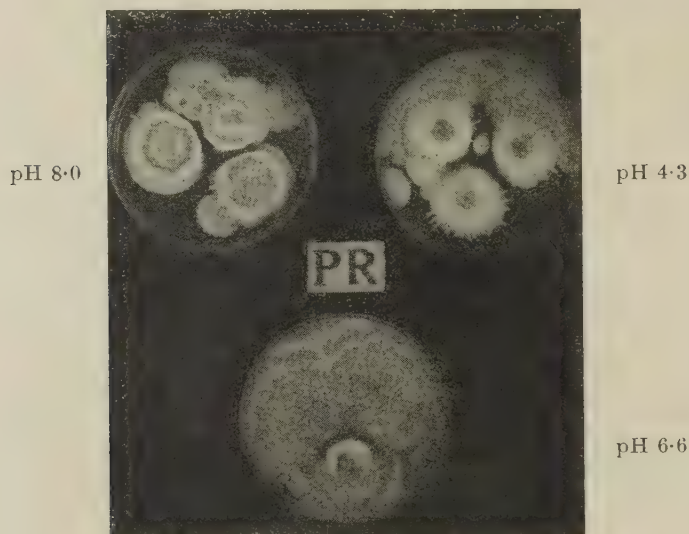


Fig. 2. Antagonism of *P. clavariaeforme* against *R. solani* (PR) at different pH values.



Fig. 3. Antagonism of organisms against *R. solani* on glucose with NaNO_3 as a nitrogen source. P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*, GN (glucose + NaNO_3).

the stimulation of growth of cellulolytic saprophytes in the soil which, being more active cellulose-destroying fungi than *R. solani*, might have suppressed the pathogen. In view of these reports, experiments were conducted to study the effect of the carbon source like glucose, starch, cel-

Table 5. Growth of the organisms on different C-sources with NaNO_3 as a N-source

Organism	Cellulose	CMC	Starch	Glucose
<i>R. solani</i>	26.8	23.3	24.0	19.2
<i>T. viride</i>	15.5	14.6	18.2	19.2
<i>P. clavariaeforme</i>	1.0	1.0	2.0	3.0
<i>C. cochlioides</i>	1.3	8.0	3.3	3.3

Table 6. Growth on different C-sources with NH_4Cl as N-source

Organism	Cellulose	CMC	Starch	Glucose
<i>R. solani</i>	20.0	15.0	11.0	12.0
<i>T. viride</i>	16.7	13.0	15.0	17.0
<i>P. clavariaeforme</i>	1.0	2.0	1.0	1.0
<i>C. cochlioides</i>	1.3	7.0	1.3	1.0

Table 8. Antagonism against *R. solani* on different C-sources with NH_4Cl as a N-source

Antagonist	Cellulose	CMC	Starch	Glucose
<i>T. viride</i>	---	—+	+	++
<i>P. clavariaeforme</i>	---	---	—+	+-
<i>C. cochlioides</i>	---	+—	++	++

Table 7. Antagonism against *R. solani* on different C-sources with NaNO_3 as a N-source

Antagonist	Cellulose	CMC	Starch	Glucose
<i>T. viride</i>	---	—+	+	++
<i>P. clavariaeforme</i>	---	---	+-	—
<i>C. cochlioides</i>	---	—+	+-	—+

lulose and sodium carboxymethyl-cellulose (CMC), with NaNO_3 or NH_4Cl as nitrogen sources, on the growth of the antagonists and the pathogen and later to investigate the antagonistic effect under these nutrient conditions.

The result of their growth in pure culture is given in Table 5 and 6.

Contrary to the general belief that *T. viride* and other saprophytes are more cellulolytic than *R. solani* (BLAIR 1941), the results definitely suggest that at least the crucifer strain of the pathogen is more cellulolytic than the other organism. Another fact is that the replacement of NaNO_3 by NH_4Cl had some retarding effect on the growth

Starch

Cellulose

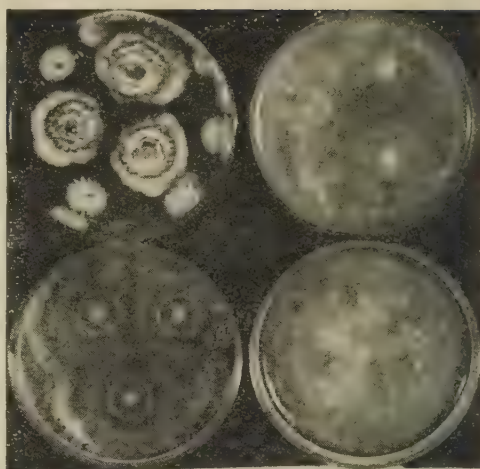


Fig. 4. Antagonism of organisms against *R. solani* on starch and cellulose with NaNO_3 as a nitrogen source. — P = *P. clavariaeforme*, C = *C. cochlioides*.



Fig. 5. Antagonism of organisms against *R. solani* on starch and sodium carboxymethyl cellulose (Gum) with NH_4Cl as a nitrogen source. — P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*.



Fig. 6. Antagonism of organisms against *R. solani* on cellulose and glucose sources with NH_4Cl as a nitrogen source. — P = *P. clavariaeforme*, T = *T. viride*, C = *C. cochlioides*.

of the organisms in general. This effect is more pronounced on the growth of *R. solani* and this agrees with the results obtained in the cultural experiments (DESHPANDE, 1956).

6. Antagonism on different C-sources (Table 7 and 8, Figs. 3, 4, 5 & 6).

In all cases cellulose did not support the antagonism of any organism against the pathogen. Glucose and starch appeared to be more favourable for the saprophytes to express their antagonism against the pathogen and the effect was more pronounced in the presence of NH_4Cl probably due to its inhibitive effect on the growth of the pathogen (DESHPANDE 1959). Although the growth in pure culture was an expression of the capacity to utilize the particular nutrient, yet it cannot be generally taken as an indicator of their antagonistic effect to the pathogen. In addition, the relatively poor growth of antagonists like *T. viride* and *C. cochlioides* and their weak antagonism to *R. solani* on cellulose was rather striking.

Acknowledgement

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Антагонизм между *Rhizoctonia solani* Kühn и некоторыми почвенными сапрофитами

BIOLOGIA PLANTARUM (PRAHA) 3 (3) : 192—199, 1961

Изучалось явление антагонизма у трех сапрофитов против патогена *Rhizoctonia solani* в разных условиях возделывания. *Trichoderma viride* и *Chaetomium cochlioides* ингибировали рост *R. solani* эффективнее при температуре инкубации 25 и 30 гр. Ц. *T. viride* антагонистически активнее в кислой среде, *T. cochlioides* в щелоческой среде. Высказывается предположение, что почвенные добавки, повышающие активность *C. cochlioides* могут быть действенным мероприятием против исследуемому патогену в щелоческих почвах. В различие от предыдущих данных культура *R. solani* целлюлотически активнее чем *T. viride* и *C. cochlioides*, что имеет место даже и в случае использования хлористого аммония в качестве источника азота.

A Method of Rapid Examination of Meiosis in Fruit-Trees

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Metoda rychlého vyšetřování meiose u jádrového ovoce

Vypracování nové metody rychlého vyšetřování meiose u jádrového ovoce bylo vynuceno rozsáhlostí materiálu, který bylo nutno zpracovat při cytologickém zhodnocení 40 sortů československého základního hrušňového sortimentu.

Metoda umožňuje během tří dnů získat trvalé řezané preparáty přibližně z 1800 prašníků.

Této metody je možno použít u všech rostlin, které mají v době průběhu redukčního dělení v pylových mateřských buňkách dostatečně velké prašníky k extirpaci.

Summary

The extent of material that had to be examined in the cytological evaluation of 40 varieties of the Czechoslovak basic assortment of pears made it necessary to work out a new, rapid method of investigation.

This method makes it possible to obtain permanent sections from about 1800 anthers within three days. During the first day, a total of three series can be imbedded in paraffin wax and each of these may contain about 600 anthers. There is sufficient time during the second day to section the three series and place the sections in gallocyanin where they are left overnight. On the following day, the sections containing about 1800 anthers are imbedded in Canadian balsam.

This method can be used with all plants having anthers sufficiently large for extirpation during the period of cell-division in the pollen mother-cells.

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Introduction

Although in recent years considerable attention has been paid to the cytological problems of pomes, no rapid method was found in the work of authors dealing with the course of reduction division or the determination of the chromosome number, for obtaining permanent microscopic preparations from a large number of anthers simultaneously.

The working out of new, more rapid methods of preparing permanent sections as described, was made necessary by the quantity of material investigated in the course of the cytological evaluation of the Czechoslovak assortment of pears, the results of which will be described in the next paper.

Method

From February to April, twigs with flower buds are taken from trees whose chromosome number is to be determined, and placed in containers of water in a cold glass-house. After a certain time, one or two buds from each examined variety are taken for testing and the stage of development of the pollen mother-cells determined by staining them with acetocarmine.

As it is frequently impossible to stain the chromosomes distinctly in some varieties without preliminary testing of various modifications of staining and also because the volume of material makes a rapid determination of its suitability for fixation necessary, the appearance of the first tetrads in the anthers of the buds may be used as a decisive criterion.

In this case, somewhat less developed buds or buds of approximately the same size are fixed, thus ensuring the presence of different stages in the development of the pollen mother-cells in the fixed material.

The part of the flower integuments covering the anthers are removed from each bud which is to be fixed, with a needle or scissors and the buds so prepared are fixed in Bouin's fluid in which the proportions of glacial acetic acid are increased to five times the amount of the original prescription.

After fixation for 24 hours, the buds are transferred to 70% alcohol in which they may be kept for a longer period. If a different fixative is chosen, it must be a mixture that will not harden the tissues of the bud too much. Excessively hardened tissues make the extirpation of the anthers difficult.

After fixation, the buds are kept in 70% alcohol which is changed several times. Then the anthers are extirpated with a needle and put into a test tube filled with 96% alcohol. The extirpation is performed as quickly as possible as the objects tend to dry up on exposure to air. The test tube is put into a shaker (HRŠEL 1952). After 10 minutes' exposure, the alcohol is replaced by fresh alcohol and the objects left in it for a further 10 minutes. Then they are put into absolute alcohol for two hours, the alcohol being replaced by fresh alcohol after the first hour. The objects are then put into a mixture of absolute alcohol and benzyl benzoate and, after the anthers have sunk to the bottom, into pure benzyl benzoate, which is changed four times within two hours. When the anthers are saturated with benzyl benzoate, the objects are sucked into a glass tube and transferred to a small box measuring $1 \times 0.7 \times 1.5$ cm., made from aluminium foil according to the following instructions:—

A shape is cut according to the attached drawing from foil 0.2 mm. thick. The dashed lines on the drawing indicate the lines to be cut. The dotted lines indicate where the foil is to be finely indented by means of a sharply pointed instrument. The planes thus formed are marked A, B, C, D, E, F, K, L and M. The plane K forms the base of the box, the planes B, E, L, M form the walls. First of all, the walls A, B, C, D, E and F are bent perpendicularly to the base K. To do this, the mentioned planes are bent along the sharp edge of a metal ruler which is placed closely to the indented dotted lines, or they are bent between the sharp edges of cutting nippers. Then the wall marked M is bent perpendicularly to the base. At the same time C and F are inserted behind B and E. The plane L is erected similarly, D is inserted in front of F and A

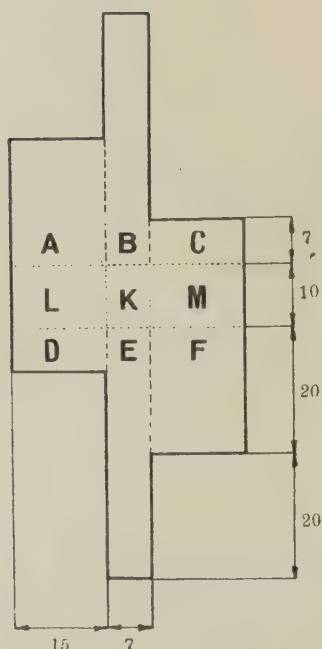


Diagram 1. Drawing of the basic figure for the making of the box. Measurements are given in millimetres.

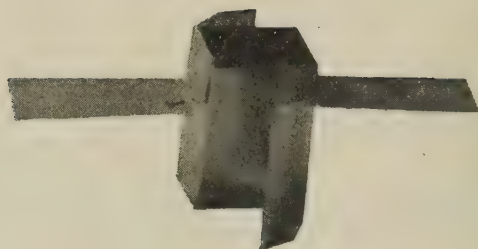


Fig. 1. Photo of the folded box.

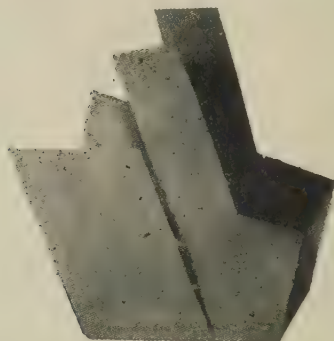


Fig. 2. A photo showing the procedure of putting up the side walls of the box.

behind C. The protruding parts of B and E are folded down over the parallel side walls K and F and pressed firmly between the flat jaws of pliers. The side walls are bent over horizontally about 5 mm. below the upper edge of the box. Similarly, parts of the planes A and F form supporting side walls which prevent the box from overturning. It must be pointed out that the planes B, E, L, M and K have to form right angles otherwise the base of the cast block containing anthers will have unequal sides and strips cut from it will be twisted sideways.

The anthers, which are transferred to the box by sucking them into a glass tube, sink to the bottom, while most of the superfluous carrier leaks out through the fine chinks at the folded edges and is absorbed by filter paper placed beneath the box. The box with the objects is then put into a small dish filled with a molten mixture of paraffin and benzyl benzoate. After changing the paraffin several times, the anthers are left in pure paraffin overnight. In the morning, the contents of the dish in which the box was placed are cooled and while the paraffin is still somewhat pliant, the box is cut out. The remaining paraffin does not stick to the aluminium foil and is easily removed in water when completely cold. When the box is opened out, a cube is obtained at the base of which, the anthers are accumulated. The block so prepared is fused to a base and can be sectioned without any further adjustment.

The advantages of this procedure are its quickness and the comparatively short exposure of the anthers to absolute alcohol which causes the objects to harden. Anthers alone saturate with paraffin much more thoroughly than when they are prepared together with the other tissues of the buds. It is also better to cut a pure suspension of anthers than a suspension of anthers with the other tissues of the receptacle hardened by their necessarily longer immersion in liquids. The benzyl benzoate need not be completely removed as it remains form, a mixture which is easily cut with paraffin. Benzyl benzoate is also not sensitive to the water contained in the 96% alcohol and binds firmly with it (Romeis). Both cutting and staining of the suspension of anthers takes less time than the processing of single buds with an equal number of anthers.

Staining with chrome lacquer of gallocylin for a period of ten to twenty hours proved to be most suitable for the examined material, or for three minutes with Ehrlich's hematoxylin to

which was added glacial acetic acid in the proportions of 5 parts staining material to from one to two parts glacial acetic acid, according to the stainability of the various species.

In varieties in which the plasm showed a higher staining power, acid or basic hydrolysis carried out after the staining of the preparations promoted decolouration and a more distinct appearance of the chromosomes. After marking the spot where a cell with suitable chromosome distribution was found in the preparation stained with galloccynin and freshly imbedded in Canadian balsam, the cover glass is removed by its immersion in xylene. The preparation is then transferred via descending alcohol concentrations to water after which it is immersed in a 1.5% solution of KOH for from 1 to 3 minutes. After washing out the alkali from the object, it is dehydrated and imbedded in Canadian balsam. It must, however, be pointed out, that in following this procedure, we risk the destruction of the preparation and the result also, may not always be satisfactory. If the duration of the immersion of the preparation in KOH is correctly estimated, for it is not always the same, then the plasm will be colourless and the chromosomes sharply outlined and of a bluish-black colour.

With preparations stained with Ehrlich's hematoxylin, hydrolysis in nHCl at a temperature of 60°C after staining, controlling and a re-immersion of the preparation in water, proved of value.

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Метод быстрого изучения мейозиса в яблонях

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Разработка нового метода для быстрого изучения мейозиса у плодовых деревьев вызвана большим количеством материала, который необходимо было обработать при цитологической оценке 40 сортов основного чехословацкого ассортимента груши.

Метод позволяет в течение трех дней получить постоянные резанные препараты приблизительно из 1800 пыльников.

Этот метод возможно использовать у всех растений, которые имеют во время редукционного деления в пыльцевых материнских клетках достаточно для экстирпации большие пыльники.

The Cytological Evaluation of Some Pear-Sorts Cultivated in Czechoslovakia

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Cytologické zhodnocení základního československého hrušňového sortimentu

Autor se zabýval cytologickým zhodnocením 40 odrůd základního československého sortimentu hrušní. Zjistil, že 30 odrůd tohoto souboru je diploidních a 10 triploidních.

Mimo stanovení chromosomálních počtů proměřil u všech odrůd i délku listových průduchů a dokázal, že je možno pomocí tohoto kritéria bezpečně odlišit diploidní sorty od odrůd triploidních.

Dalším rozlišovacím znakem je u triploidních sort zaostávání chromosomů ve vřeténku během anafáze heterotypického dělení. Z provedeného zhodnocení průběhu redukčního dělení vyplynuly některé nové poznatky týkající se abnormálního průběhu meiose u diploidních a triploidních odrůd.

Autor našel u jednotlivých sort rozdílnou barvitelnost pletiv, která nezávisela na způsobu mikrotechnického zpracování, nýbrž byla především vlastností každé odrůdy.

Dále doplnil údaje o výši klíčivosti pylu u sort, u nichž dosud nebyla v klimatických podmínkách Československa stanovena.

Summary

The author carried out a cytological evaluation of 40 varieties of the basic Czechoslovak assortment of pears. Thirty of these were diploid and ten were triploid.

In addition to the chromosome number of these varieties, he determined the average dimension of the leaf stomata, to prove that it is possible to use this method in pear trees to make a reliable differentiation between diploid and triploid varieties. From a determination of the average lengths

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of the stomata it follows that the diploid varieties of pears can be distinguished from the triploid varieties by means of this criterion.

A further, very accurate mark of difference between diploid and triploid varieties, is the lagging of chromosomes in the spindle during the anaphase of the heterotypical division.

In the various varieties a divergent susceptibility to staining of the tissues was found which is not dependent on the method of microtechnical processing but in the first place, on the properties of each individual variety.

Regarding the statements of Darlington and Moffet on the one hand and Sax's objections on the other, the author's cytological findings in the case of triploid varieties are in agreement with those of Sax and also with his opinion on the debatableness of polyvalent associations found by Darlington and Moffet.

Introduction

In recent years considerable attention has been paid to the determination of the cytological characteristics of Kernel-fruit and especially of their practical application.

AUCHTER (1921), BEAUMONT and KNIGHT (1922), KOBEL (1924), KAMLAH (1929), BRANSCHIED (1929), RUDLOFF (1938) and SCHANDERL (1938) examined the pollen germination, RYBIN (1926), KOBEL (1926), SHOEMAKER (1926), NEBEL (1929), NATIVIDADE (1932) and MOFFET (1934) investigated the chromosome number and the course of meiosis. On the basis of the observation of secondary associations formed during reduction division, DARLINGTON and MOFFET (1930) and later SAX (1932) gave their opinions regarding the genesis of *Pomoideae*.

A collective cytological evaluation of the basic Czechoslovak assortment of pears, comprising a total of 40 varieties, was worked out. In addition to the determination of their chromosome numbers, the germination of pollen was also ascertained in species in which it has not been determined under the conditions prevailing in Czechoslovakia. In this way, the work of ZVOŇÍČKOVÁ-SOSNOVÁ (1949) who had investigated the germination of pollen in the majority of the Czechoslovak assortment of pears, was continued. The average size of the stomata in the leaves of all examined varieties was also determined with regard to the possibility of in this way ascertaining the grade of polyploidy. Attention was also paid to the process of meiotic division in diploid and triploid varieties so as to be able to assess the findings of the authors DARLINGTON, MOFFET and SAX already mentioned.

Material and Methods

Cytological evaluation was carried out in the following varieties:

Ananas de Courtray, Bergamotte Esperen, Beurré Alexandre Lucas, Beurré Amanlis, Beurré Baguet, Beurré Bose, Beurré d'Anjou, Beurré Diel, Beurré Hardy, Beurré Superfine, Bonne d'Ezée, Citron des Carmes, Clapp's Favorite, Colorée de Juillet, Comtesse de Paris, Conference,

Conseiller de la Cour, Doyenné du Comice, Doyenné d'Hiver, Doyenné de Mérode, Fondant Thirriot, Grise Bonne, Joséphine de Malines, Madame Verté, Minister Dr. Lucius, Musqué de Bohême, Musqué de Turque, Napoleons Butterbirne, Nouveau Poiteau, Poire de Solany, Précoce de Trévoux, Président Drouard, Président Mas, Rousselet d'Stuttgart, Salisbury, Suprême Coloma, Vicar of Winkfield, Williams Bon Chrétien, Winter Nellis, Fondante de Charneu.

The determination of the chromosome numbers was made from the pollen mother-cells in which reduction division was taking place and from the tissues of developing leaves. Rapid cytological methods of staining with acetocarmine and nigrosin and a method enabling a quicker preparation of cut sections from a large number of anthers simultaneously was used.

This method was worked out by the author especially for the purpose and it was described in an earlier paper in this journal.

The methods employed by Zvoníčková-Sosnová were used in examining the germination of the pollen. An application of the same working procedure makes it possible to complement the data concerning the germination of those varieties for which it had not been determined by the above author. Methods used at the Department of Genetics at the Faculty of Natural Sciences of Charles University were used to determine the length of the stomata of the leaves.

Five, already mature leaves, were selected from each variety and preparations made from them. From these, one preparation was chosen in which 100 stomata were measured.



Fig. 1. An unusual division of the end of the pollen tube into two parts in Williams Bon Chrétien

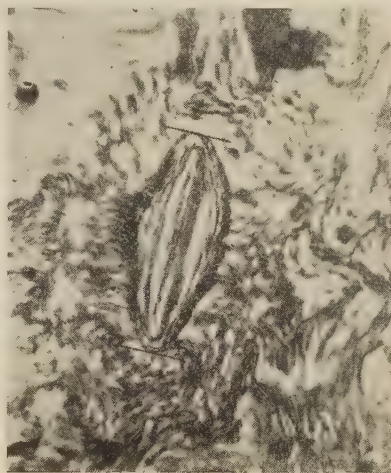


Fig. 2. Photo of the stomata of the pear tree leaf. In the photo the measured length of the stoma is shown in black lines.

In order to distinguish the diploid varieties from the triploid varieties, it was quite sufficient to determine the size of the stomata of only one leaf. Determination of the average dimensions of the stomata as a characteristic of the various varieties was not intended. For this reason, no attention was paid to variability among the leaves.

Already the first measuring showed that the various parts of the leaf blade may have stomata of considerably differing dimensions. In the biometric evaluation of the measured lengths of the stomata from the base, the middle and from the top of the blade of one leaf performed with two varieties (Williams Bon Chrétien and Doyenné de Mérode), a significant difference was found between the averages obtained from the base and those from the centre of the blade. In the case of Williams Bon Chrétien the difference between the base and the top lies on the borders of significance. In the case of Doyenné de Mérode it is significant. In the case given, the results obtained by measuring the stomata from the centre and top can be considered identical.



Fig. 3. A graphic proof of the possibility of distinguishing diploid varieties from triploid varieties of pears. Triploid varieties have a longer average length of the stomata than diploid varieties. Length of stomata in microns. Species arranged according to the graduated length of their stomata. Diploid varieties ————— Triploid varieties - - - - -

With regard to the difference between corresponding parts of the leaves of both varieties, differences in the averages of all corresponding parts are highly significant. Therefore, in order to determine the variability of all three parts of the leaf, the F test for comparing variances was carried out. The results obtained showed that no part of the leaf shows any statistically significant differences as regards variability.

Hence in all varieties, the stomata of the middle part of the leaf blade were evaluated from the section approximately halfway between the central nerve and the edge of the leaf, as in this section not only was the highest relative difference between the varieties found, but also it is the easiest part to work with. Because these results, in the extent of their variability, are no different from those of other parts, their use is justified.

The longest dimension of the stomata was not measured, but the part shown in the photograph of the pear tree leaf stoma, since this dimension is less dependent on the degree of opening of the guard cells and because the wrinkled surface of the cuticle makes the determination of their length difficult. In the case of small stomata, the wrinkled surface frequently makes it impossible to decide whether they are closed or whether the wrinkling gives such an impression. Hence, for the evaluation, only stomata as could be determined definitely were taken into consideration. It is also possible that metacrylate shows smaller stomata less accurately than larger and normally developed ones. Therefore, in the case of some varieties which had a greater number of such stomata, Ananas de Courtray, Beurré Superfine, Doyenné du Comice, Musquée de Turque, Rousselet d'Stuttgart, Vicar of Winkfield, the averages could appear to be somewhat higher.

Results and Discussion

1. The meiosis and the chromosome number

On the basis of the author's findings with diploid varieties, it can be confirmed that the course of reduction division in pear trees which is known in detail, certainly shows no substantial abnormalities. This is shown by the fact that only a few anomalous tetrads were found in the examined material. In material from the season 1958 of the Ananas de Courtray variety, only a few pentads were found and in the variety Citron des Carmes, single tetrads in which three cells were developed normally while the fourth cell was smaller and crescent-shaped.

Special attention was paid to the varieties President Mas and Nouveau Poiteau which show a low pollen germination. In contrast to the other diploid varieties, metaphasic plates of both the first and second metaphase were

found in which considerable dispersion of single chromosomes could be observed. In most cases, the chromosomes were not spread in one plane but were dispersed in all directions as can be observed in some triploid varieties. Single chromosomes were some distance from the periphery of the plate. In the plates of the first metaphase which were very clear, single bivalents sometimes quite far apart were observed. Orientation of the metaphasic plates in the second metaphase may be very varied. Both plates are very close together and in extreme cases, even touching. The occurrence of one larger and one smaller plate in one cell was also observed. In single cases pentads consisting of two cells of normal shape and size, two cells of atypical cylindrical shape and one small cell containing a small nucleus not in the centre of the cell but in its protracted part, were also found. In rare cases tetrads smaller by a fourth, were found.

In no diploid varieties, however, were any lagging chromosomes found in the middle of the dividing spindle.

Far more abnormal tetrads were observed in triploid varieties which indicates an abnormal course of the meiosis. It was found that the occurrence of an abnormal number of tetrads is not constant in one variety in single flowers at different seasons. In some varieties, especially *Beurré Diel*—season 1959, anomalous tetrads were found only in small numbers. In triploid varieties tetrads made up of five cells and even cases of six and seven cells were most often found besides normal tetrads. In five-cell tetrads, one of the cells is usually considerably enlarged at the expense of the others. It is always of a regular, rounded shape whereas the other cells are cylindrical. These tetrads also, do not have the normal shape. One of the cells is usually very small and contains a small nucleus. In tetrads consisting of six or seven cells, from two to four very small cells are usually formed.

In *Beurré Diel* a triad was found and in *Musquée de Bohême* a diad. It can be said generally that tetrads and young pollen mother-cells of triploid varieties are somewhat larger than in diploid varieties. This is true for the tetrads of *Beurré Alexandre Lucas*, *Conseiller de la Cour*, *Musquée de Bohême* and *Beurré Baguet*.

During the course of reduction division in triploid varieties, all abnormalities described in the references were found. Only in a few anaphasic figures of the first reduction division, was no lagging of the chromosomes in the axis observed. Similarly, during the course of the second anaphase, a lagging of single valents was confirmed, rarely of course, when compared with the average amount of univalents lagging in the anaphase of heterotypical division, which averages more than eight. The metaphases of reduction division are often quite indistinct as the chromosomes form groups which make it impossible to observe all of them. In heterotypical metaphasic plates from a lateral view, single univalents were often found a considerable distance in the direction of the poles from the remaining chromosomes arranged in an equatorial plane. The appearance of the metaphase in those plates where no single removed chromosomes were found was also very irregular as compared with the configuration of diploid varieties, as has already been stated. Homeotypical metaphases observed from the side had a more regular appearance.

Table 1. The chromosome numbers of the examined varieties

Diploid varieties ($2n = 34$)
Ananas de Courtray, Bergamotte Esperen, Beurré Bosc, Beurré d'Anjou, Beurré Hardy, Beurré Superfine, Bone d'Ezée, Citron des Carmes, Clapp's Favorite, Colorée de Juillet, Comtesse de Paris, Conference, Doyenné d'Hiver, Doyenné du Comice, Fondante de Charneu, Fondant Thirriot, Josephine de Malines, Madame Verté, Musquée de Turque, Napoleons Butterbirne, Nouveau Poiteau, Poire de Solany, Précoce de Trévoux, Président Drouard, Président Mas, Rousselet d'Stuttgart, Salisbury, Suprême Coloma, Williams Bon Chrétien, Winter Nellis
Triploid varieties ($2n = 51$)
Beurré Alexandre Lucas, Beurré Amanlis, Beurré Baguet, Beurré Diel, Conseiller de la Cour, Doyenné de Mérode, Grise Bonne, Minister Dr. Lucius, Musquée de Bohême, Wicar of Winkfield

It is very difficult to distinguish between bivalents and trivalents in such metaphases. A definite differentiation is possible only by means of very thin slices in which only part of the chromosomes remains in the pollen mother-cell. In such cases, only bivalents were observed in diploid varieties. Trivalents in triploids, as far as could be seen distinctly, were of the Y type. However, as not many cells were suitable for the certain differentiation of bivalents and trivalents, no conclusion regarding their incidence can be arrived at. This is in agreement with SAX's 1932 objection to DARLINGTON and MOFFET's 1930 findings, that in these cases, it is very difficult to distinguish with certainty between possible polyvalents and configurations of chromosomes forming secondary associations. At the most, it can be said also that MOFFET's (KOBEL 1954) observations regarding the occurrence of trivalents, are based on facts.

In some triploid varieties too, plates of heterotypical metaphase were found in the polar projection consisting of only bivalents and univalents spread out quite evenly in the centre of the pollen mother-cell without any indication of association with chromosomes. This confirms the comparatively rare occurrence of trivalents in triploid varieties.

The occurrence of cells with two or more nuclei and their relation to the chromosome number was also observed. Single cells with more than one nucleus were found, however, in both diploid and triploid varieties.

From a study of reduction division in the examined assortment of pear trees, it follows that apart from the determination of chromosome numbers as given in Table 1, the lagging of chromosomes in the spindle during the heterotypical anaphase may also be used as a criterion for distinguishing diploid from triploid varieties. To make an estimation based on the tetrads, the size of the pollen grains and the occurrence of anomalous tetrads, is inaccurate and may lead to errors.

The determination of the chromosome numbers in the examined material gives further proof that it is possible to find a very different susceptibility

Table 2. The percentage of germinability of pollen of the examined varieties

	$\bar{x}$	s_x	$s_{\bar{x}}$
Ananas de Courtray	55.86	4.37	1.96
Bergamotte Esperen	50.41	2.41	1.80
Beurré Alexandre Lucas	4.64	2.15	0.96
Beurré d'Anjou	51.51	1.81	0.81
Beurré Superfine	79.45	4.41	1.97
Bonne d'Ezée	72.53	4.24	1.90
Doyenné d'Hiver	75.37	4.72	2.12
Doyenné de Mérode	5.25	1.21	0.53
Fondante de Charneu	68.52	5.93	2.70
Grise Bonne	17.45	1.71	0.76
Josephine de Malines	48.12	3.29	1.47
Musquée de Bohême	3.64	0.78	0.35
Musquée de Turquie	65.68	2.56	1.14
Précoce de Trévoux	71.63	5.36	2.39
Président Mas	34.64	2.35	1.05
Rousselet d'Stuttgart	65.71	2.61	1.17
Suprême Coloma	83.37	1.32	0.58

to the staining of tissues in various varieties. This was already shown in the results of staining with nigrosin and acetocarmine. Whereas in some varieties a contrasting staining of the chromosomes and plasm was achieved without any special adjustment, in other varieties the high susceptibility of the plasm to staining was remarkable especially during the course of reduction division. The plasm of young pollen mother-cells of all varieties stained with the same intensity, but with far less intensity, when compared with pollen mother-cells in which reduction division was progressing.

The staining of sections of permanent preparations confirms this fact. Although all material was fixed and processed in the same way, the fixation picture and the susceptibility to staining of the tissues sometimes differed. Other authors also found that after fixing material from different varieties of pear trees with the same fixation mixture, they obtained a varied fixation picture. They could not call attention to the difference in susceptibility to staining, however, as they used regressive staining in all cases.

2. Germinability of the pollen

The data regarding the germinability of pollen are given in Table 2. Upon examination of preparations from the Williams Bom Chrétien variety, an unusual division of the end of the pollen tube into two parts was found in one case.

3. The stomata

The results of measuring 4000 stomata which are given in Table 3, show that this method may be applied successfully for the differentiation of diploid and triploid varieties of fruit trees. Contrary to the expected wider variability

Table 3. The average length of stomata expressed in microns (s_x^- is always ten times smaller than s_x , as the average lengths were calculated from 100 measurements)

Diploid varieties					
	$\bar{x}$	S_x		$\bar{x}$	S_x
Président Mas	31.84	4.85	Winter Nellis	33.31	5.44
Madame Verté	32.27	4.84	Beurré Bose	33.36	4.30
Ananas de Courtray	32.31	5.37	Président Drouard	33.44	4.78
Beurré d'Anjou	32.60	3.98	Napoleons Butterbirne	34.04	5.15
Fondant Thirriot	32.63	4.01	Bergamotte Esperen	34.10	5.15
Conference	32.75	3.81	Williams Bon Chrétien	34.22	5.58
Rousselet d'Stuttgart	32.77	4.99	Colorée de Juilliet	34.38	4.73
Doyenné de Comice	32.83	4.99	Citron des Carmes	35.20	3.26
Doyenné d'Hiver	32.89	3.95	Salisbury	35.22	5.58
Beurré Hardy	32.91	4.18	Précoce de Trévoux	35.25	4.50
Beurré Superfine	33.03	4.13	Poire de Solany	35.46	3.97
Bonne d'Ezzée	33.14	4.80	Comtesse de Paris	35.53	4.06
Nouveau Poiteau	33.20	4.74	Josephine de Malines	35.80	3.73
Fondante de Charneu	33.20	3.25	Clapp's Favorite	35.96	4.13
Musquée de Turquie	33.22	4.45			
Suprême Coloma	33.27	3.78			
Triploid varieties					
Beurré Amanlis	36.67	5.57	Beurré Baguet	38.35	5.02
Beurré Alexandre Lucas	37.15	4.51	Beurré Diel	39.36	4.91
Doyenné de Mérode	37.17	4.37	Musquée de Bohême	40.19	5.68
Minister Dr Lucius	37.66	4.92	Grise Bonne	42.16	4.75
Vicar of Winkfield	37.84	5.39	Conseiller de la Cour	42.73	4.46

in triploid varieties, it was found that variability in some diploid varieties is considerably wider than in the variety Doyenné de Mérode, which is the triploid having the most narrow variability.

It was found that examination of the average size of stomata could provide very valuable and reliable information on the chromosome numbers. Without checking the chromosome number, the diploid variety Marguerite Marillat might be ranked among triploid varieties because of the low germinability of its pollen, but a checking of the size of its stomata, an average of $\bar{x} \pm 3 \cdot s_x = 34.29 \pm 3 \cdot 0.50$, definitely places it among diploids.

Consequently, on the basis of the average dimensions of the stomata and taking the pollen germinability into consideration, it is possible to determine the grade of polyploidy with sufficient accuracy. It is necessary to take into account the data on germinability only in the case of varieties in which the average dimensions of stomata are close to those of triploid varieties.

Varieties having germination of around 30% can be safely distinguished by the average dimensions of stomata. Examples of such varieties are Nouveau Poiteau, Président Mas and Winter Nellis which, according to the percentual result of their pollen germinability, are ranked in the same group as triploid

varieties [ZIEGLER and BRANSCHIEDT (1927), BRANSCHIEDT (1929) and SCHANDERL (1938),] whereas the average dimensions of their stomata would place them among diploid varieties where they actually belong.

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Цитологическая оценка основного чехословацкого ассортимента груши

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Автор дает цитологическую оценку 40 сортов основного чехословацкого ассортимента груши. Им установлено, что 30 сортов этого набора диплоидных и 10 триплоидных.

Кроме установления хромосомных чисел измерялась у всех сортов и длина устьиц листьев. Доказано, что при помощи этого критерия возможно вполне надежно отличать диплоидные сорта от триплоидных.

Дальнейшим признаком для разграничения триплоидных сортов является запаздывание хромосом в течение анафазы гетеротипического деления. Из приведенной оценки течения редукционного деления вытекают, новые данные относительно ненормального течения мейозиса у диплоидных и триплоидных сортов. Автором найдены у отдельных сортов различия окрашиваемости тканей, которая не зависит от способа микротехнической обработки, а является прежде всего свойством каждого сорта.

Дальше дополнены данные о прорастании пыльцы сортов, для которых в климатических условиях Чехословакии пока не установлены.

Transmission of *Marmor cichorii* KvíčALA to *Chenopodium giganteum* DON.

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Přenos viru skvrnitosti čekanky na *Chenopodium giganteum* DON.

Virus skvrnitosti čekanky (*Marmor cichorii* KvíčALA) byl přenesen na *Chenopodium giganteum*, na němž vyvolává charakteristické nekrotické leze. K očkování bylo užito cysteinhydrochloridu a karborunda. Virus se nezdařilo přenést na okurku ani na *Amaranthus caudatus*.

The chicory blotch and yellows virus (*Marmor cichorii* KvíčALA) was hitherto transmitted to *Chenopodium Quinoa* WILLD. in which the virus caused local lesions very similar to those caused by the common cucumber mosaic virus (BRČÁK, 1960). Further attempts made with the same material showed that the infection of *Ch. Quinoa* resulted in local lesions only and no systemic infection was observed in glasshouse conditions. Attempts to transmit the virus mechanically (using carborundum) from the lesions on *Ch. Quinoa* to cucumber seedlings as well as to *Amaranthus caudatus* L. failed. Negative results were obtained from rubbing cucumber cotyledons with crude sap taken from diseased chicory leaves.

In our further experiments carried out in order to transmit the virus from chicory to some test plants, cysteine hydrochloride in concn. of 0.02M was added to the inoculum (HOUGAS, 1951) and the test plants were kept 24 hrs. in darkness and their tops were cut off before the inoculation. In all cases carborundum (600 mesh) was used. Positive transmissions were obtained from rubbing halfleaves of *Chenopodium giganteum* DON. Large local lesions were formed reaching 2—3.5 mm. in diameter within twenty days (at an average min. temperature of 9.6° C, max. 35.8° C and at an average max. air humidity of 97%, min. 41%). Sharp bordered lesions had a pale brown centre and an reddish brown border dissolving slightly in a small chlorotic ring outside of the lesion. No systemic infection was observed in *Ch. giganteum*. Under the same experimental conditions the infection of *Ch. Quinoa* was characterized by large orange lesions formed both in green and in decolorized old leaves. The control halfleaves rubbed in the same manner with sap taken from healthy chicory plants showed no reaction. Attempts to transmit the virus from the crushed lesions of *Ch. giganteum* to cucumber and to *A. caudatus* gave negative results.



According to comparative experiments with some strains of the common cucumber mosaic virus carried out in the same time, it is suggested that the virus of chicory blotch and yellows described by KVIČALA (1956) is not a strain of the common cucumber mosaic virus.

Fig. 1. Local lesions on the leaf of *Chenopodium giganteum* by the chicory blotch and yellows virus twenty days after inoculation.

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Перенос вируса пятнистости цикории на *Chenopodium giganteum* Don.

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Вирус пятнистости цикории (*Marmor cichorii* Kvičala) был механически перенесен на *Chenopodium giganteum* Don., на листьях которого он вызывает местные некрозы. Вирус удалось перенести при помощи цистенилгидрохлорида и карборунда. Инкуляция огурцов и *Amaranthus caudatus* этим вирусом дала отрицательные результаты.

The Use of the Quantitative Ring Precipitation Reaction for Determining the Intensity of Cross Reactions with Special Reference to Comparative Serology

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Stanovení intenzity zkřížených reakcí pomocí kvantitativní prstěncové precipitace

V práci jsou uvedeny praktické pokyny a příklady na použití kvantitativní prstěncové precipitace ke stanovení intenzity zkřížených reakcí. Byla ověřena schopnost této metody objektivně reprodukovat intenzity reagujících systémů antigen—protilátka za různých koncentračních poměrů. Bylo provedeno porovnání Boydenovy metody „úplných křivek“ a metody kvantitativní prstěncové precipitace.

Výhodou použití kvantitativní prstěncové precipitace je úspora antisera (na jedno stanovení stačí 0.05 ml antisera), menší náročnost na pracovní čas a patrně menší citlivost na nespecifické faktory.

Kvantitativní prstěncová precipitace (KPP) je vhodnou metodou pro kvantitativní experimenty v imunochémii a pro účely srovnávací serologie.

Summary

Practical directions and examples are given for the use of quantitative ring precipitation to determine the intensity of cross reactions. The ability of this method to reproduce objectively the intensity of an antigen-antibody reacting system under various concentrations was verified. A comparison was made between Boyden's method of "complete curves" and the quantitative ring precipitation method.

The advantage of using quantitative ring precipitation is the saving of antiserum (0.05 ml. is sufficient for one determination), the smaller demand

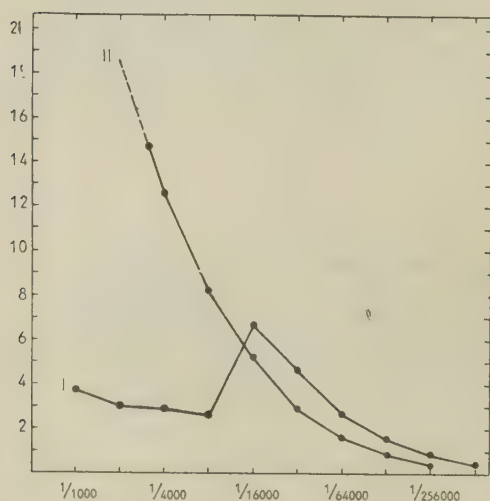
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on working time and a noticeably reduced sensitivity to non-specific factors.

Quantitative ring precipitation (QRP) is a suitable method for quantitative experiments in immunochemistry and for purposes of comparative serology.

Introduction

The present paper is a continuation of the work carried out by KLEINE, MATTHES, MÜLLER 1957, KLEINE, MÜLLER and MATTHES 1958 and especially of the work in which the objective evaluation of the intensity of the ring precipitation (layering) reaction was used for the determination of the amount of antigen (KLOZ 1960b). Methodical details are also described there.



Graph 1. Comparison of intensities: I. ordinary precipitation reaction in which antigen and antiserum are mixed. II. Ring precipitation reaction in which antiserum is layered with antigen with various dilutions of antigen. Details in text. x : dilution of antigen in geometrical series (the graph is laid out according to the custom of Boyden's school y : turbidity according to the galvanometer scale; measured with a photronreflectometer. Curve I: Normal precipitation reaction was carried out in cuvettes 5 mm. thick. For each reaction 0.1 ml. antiserum against crystalline phaseolin (protein was isolated from seed of the species *Phaseolus vulgaris* L.) was mixed with 1.9 ml. antigen phaseolin suitably diluted. Incubation: 40 minutes at laboratory temperature. Curve II: Ring precipitation reaction. 0.05 ml. antiserum against crystalline phaseolin was layered for each reaction with 0.65 ml. solution of crystalline phaseolin suitably diluted. Incubation: 40 minutes at laboratory temperature.

In the author's previous paper (KLOZ 1960b), it was shown that the intensity of the ring precipitation reaction in a homologous reaction at a relatively wide range of antigen concentration is directly proportional to the amount of antigen (see graph 1 — using rabbit serum under given conditions).

If it is quite possible, as shown in the author's paper mentioned previously, to determine the amount of antigen by the use of quantitative ring precipitation (QRP) in homologous systems, then it is necessary in cross reactions using the same quantity (concentration) of antigen to attribute differences in the intensity of the reactions to the different qualities of these antigens.

This leads to the idea that with quantitative ring precipitation (as the objective evaluation of the intensity of ring precipitation reaction is now called), it would be possible to avoid Boyden's requirement of "complete

curves" during the determination of the intensity of cross reactions (this requirement is fully justified in otherwise normal precipitation reactions in which antigen and antiserum are mixed together — cf. BOYDEN 1942, BOYDEN, DEFALCO 1943, BOYDEN 1954, MORITZ 1960).

In the present work, the value of QRP was confirmed in relation to the objective reproduction of the intensity of reacting antigen-antibody systems under various quantitative conditions and in comparison with Boyden's method.

Plant proteins were used in the reaction systems described below as they are under investigation in our laboratory. As antigens, plant proteins are in general equal in value to proteins of animal origin and in this respect, there is no difference between them. This method can be used therefore with the same effectivity for proteins of other than plant origin.

Material and Methods

1. Determination of the intensity of reacting systems under different concentrations of antigen and antiserum

Protein from the seeds of peas (*Pisum sativum* POIR), the sort "Dětenický yellow large-grained" was used, i.e.

- a) fractions soluble in water (albumin fractions)
- b) globulin legumin

The albumin fraction was obtained by extraction of 100 g. of flour from pea-seeds with 500 ml. 1 M NaCl. After filtration, the extract was saturated to 70% with ammonium sulphate, the coagulated protein was filtered off by suction using Büchner's funnel, the cake broken up and again dissolved in 200 ml. 0.2 M NaCl. Dialysis was carried out first of all against running tap water for 48 hours and then for 24 hours in the cold against repeatedly changed distilled water, the fraction was centrifuged, then filtered through a folded filter in the cold. The filtered supernatant containing in the main albumin fractions, was lyophilised.

Globulin legumin was isolated from the same flour according to DANIELSSON (1950).

Antiserum was obtained from rabbits immunised:

a) with albumin fractions: the rabbit was immunised with 24 intravenous injections during a period of 81 days (5 of these were with alum adjuvants), altogether 111 mg. of proteins. The titre rose slowly.

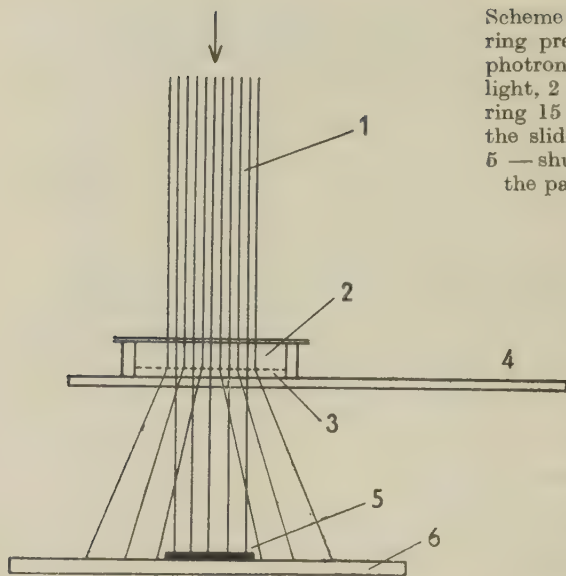
b) with legumin: the rabbit received 12 intravenous injections during a period of 42 days, altogether 28 mg. of antigen. Serum was obtained from both rabbits in the normal way and was lyophilised.

Diagnostic sera were prepared by dissolving lyophilised preparations in a 7%, 14%, 21% and 28% solution (w/w), in the case of turbidity it was centrifuged at about 15,000 g.

The antigens and antisera were evacuated for a short time immediately before use in the receiver of a one-stage rotary pump to minimise the formation of bubbles.

The reactions were carried out in cuvettes as described in the earlier work (KLOZ 1960b). In each case, 0.05 ml. of antiserum was pipetted on the bottom of the cuvette and overlaid carefully with antigen. (This was layered with a Pasteur pipette onto the upper plane of the glass ring of the cuvette.) The cuvette was covered with a cover slip and after 40 minutes, incubation at a laboratory temperature of about +20°C, the degree of precipitation was determined by the use of a vertically installed photorefractometer (LIBBY 1938, KLOZ 1960a).

Libby's photorefractometer is a relatively simple and sensitive apparatus: only rays of light deflected from the parallel bundles are measured with this apparatus (only dispersed light).



Scheme No. 1. Principle of measuring intensity of ring precipitation reaction by the aid of Libby's photoreflectometer. 1 — bundle of parallel rays of light, 2 — antigen, 3 — antiserum, 4 — cuvette (glass ring 15 mm. in diameter and 4 mm. high is fixed to the slide; after filling is covered with a cover slip, 5 — shuttered dark spot on photoelectric cell (opposite the parallel bundle of rays), 6 — photoelectric cell.

In the present case, the originally parallel ray of light is dispersed on the film of the precipitate which forms on the dividing line between antiserum and antigen layers. (See scheme 1.)

Libby's photoreflectometer was set by a fixed standard so that a variation of 10 on the scale of the galvanometer corresponded to McFarland's turbidity No. 1 (99 ml. 1% H_2SO_4 + 1 ml. 1% BaCl_2). Reproducibility was tested between readings by means of a fixed standard. The turbidity value of the antiserum itself is subtracted from the final values of turbidity of the specific reaction measured by covering 0.05 ml. antiserum in the cuvette with physiological saline and subtracting the turbidity after 40 minutes.

When new, unknown antigens are used, or antigens in higher concentration, it is necessary to test them for the presence of non-specific factors: A normal serum of the same concentration as the antigen is prepared and possible values of non-specific reactions are determined (again after subtracting the turbidity of the pure normal serum). Possible turbidity of pure antigen is already included. These values of non-specific reactions obtained by the aid of normal serum are subtracted from the values obtained with antiserum to give the precise values of the specific reactions.

In order to avoid individual differences in sera, it is better to take normal serum from rabbits before immunisation and work with antiserum and normal serum from one and the same rabbit.

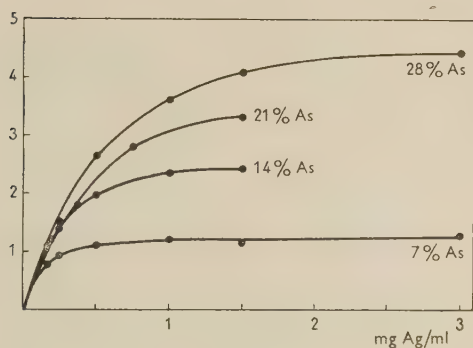
Every value given in the tables is an average of two parallel reactions. Tab. 1 and graphs 2 and 3 give the values of the reaction system albumin fraction from pea-seed x antiserum against albumin fraction from pea-seed. Tab. 2 and graphs 4 and 5 give the values of the reaction system legumin x antiserum against legumin.

Table 1. Details in text

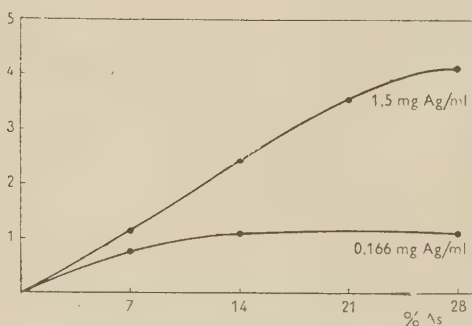
Turbidity of reactions									
	Concentration of antigen in mg./1 ml.								
	0.166	0.187	0.25	0.375	0.5	0.75	1.0	1.5	3.0
Ag*) albumin x 28% As*) against albumin	1.08		1.54		2.66		3.63	4.09	4.44
x 21% As against albumin		1.20		1.81		2.81		3.34	
x 14% As against albumin	1.09		1.38		1.98		2.35	2.43	
x 7% As against albumin	0.76		0.93		1.11		1.21	1.14	1.28

2. Determination of the intensity of cross reactions under varied antigen concentrations

The taxonic specificity of proteins from several kinds of plants under varied antigen concentrations was determined and the extent to which the differentiating power of the antiserum used changes at the same time.



Graph 2. Graphical expression of values from table 1. Details in text. x : antigen concentration, y : turbidity



Graph 3. Graphical expression of values from table 1. Details in text. x : antiserum concentration, y : turbidity.

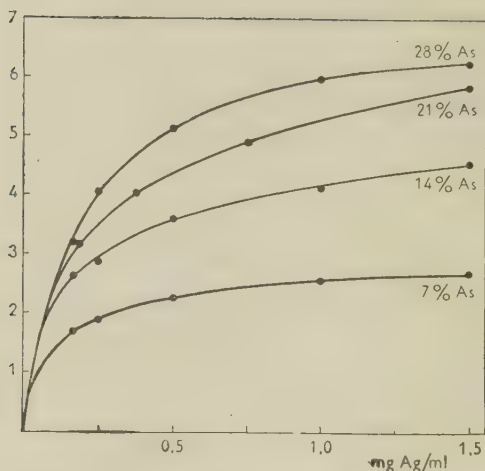
Antigenic complexes of structural proteins extracted from the sub-cotyledoneous part of seedlings (the hypocotyl and the root) were used as antigens. "Acetone" preparations in powder form were used for extraction. They were prepared by homogenising the plant parts in cooled acetone with the addition of MgO followed by rinsing in cooled ether and drying of the preparations. (For the use of these preparations in comparative serology of plants, see KLOZ 1960a.) Preparations from the given organs of the species *Phaseolus vulgaris* L. (Dětenická Konserva variety), *Phaseolus aureus* L., *Glycine soya* [L.] STEB. ZUCC. (Nord-Deutsche schwarzmatte variety), *Pisum sativum* POIR. (Dětenický yellow large-seed variety), *Lupinus luteus* L. were extracted with physiological saline at pH 7.3. The proteins in the centrifuged extract were determined accurately by the biuret reaction (after precipitation with trichloroacetic acid and redissolving

Table 2. Details in text

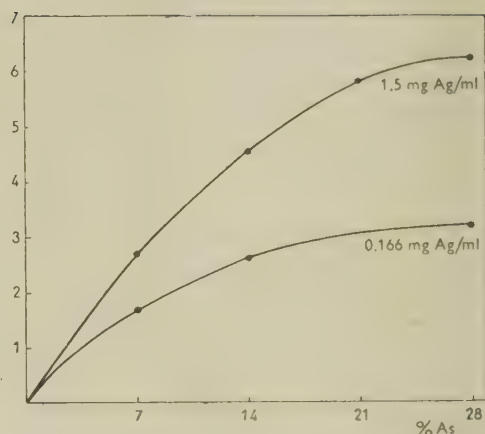
Turbidity of reactions									
	Concentration of antigen in mg./1 ml								
	0.166	0.187	0.25	0.375	0.5	0.75	1.0	1.5	3.0
	0.166	0.187	0.25	0.375	0.5	0.75	1.0	1.5	3.0
Ag*) legumin									
x 28% As**) against legumin	3.20	3.16	4.06	4.04	5.13	4.91	5.98	6.23	
x 21% As against legumin	2.63		2.88		3.65		4.13	5.84	
x 14% As against legumin	1.70		1.90		2.27		2.57	4.55	
x 7% As against legumin								2.70	

*) Ag = antigen

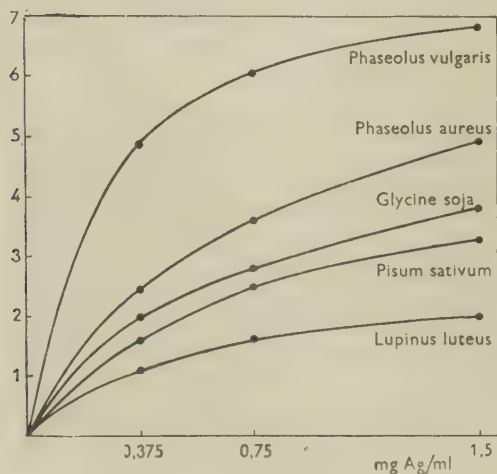
**) As = antiserum



Graph 4. Graphical expression of values from table 2. x : antigen concentration, y : turbidity.



Graph 5. Graphical expression of values from table 2. x : antiserum concentration, y : turbidity.



Graph 6. Graphical expression of values from table 3. x : antigen concentration, y : turbidity

in 1 N NaOH — modification of LEWIN and BRAUER 1951). The extracts were then diluted to basic concentrations of 1.5 mg. proteins to 1 ml.*).

After diluting, these solutions were used for the test.

The antiserum was prepared by immunising a rabbit with an antigen complex from the sub-cotyledoneous part of the seedling *Phaseolus vulgaris* L., nanus (Dětenická Konzerva sort). During a period of 48 days, the rabbit received 15 intravenous injections containing a total of 180 mg. proteins. Antiserum was obtained in the usual way, lyophilised and a 28% solution in distilled water (w/w) made from the lyophilised preparations and centrifuged. The reactions were carried out in the usual way as in the previous experiment.

Results are given in tab. 3 and graph 6.

3. Comparison of the results obtained by Boyden's method of "complete curves" and those of QRP

Boyden's method is a suitable and already adequately tested method for purposes of comparative serology (see for instance BOYDEN 1942, BOYDEN, DEFALCO 1943, BOYDEN 1954, MORITZ 1960 and others). We have used Boy-

*) It is better to dilute the solution to a concentration of 2.0 mg. protein to 1 ml. after determining protein by the biuret reaction, than to determine proteins once more in the same way (for control) and dilute the solution definitely to 1.5 mg./ml.

Table 3. Details in text

Turbidity of reactions			
	Concentration of antigen in mg./l ml.		
	0.375	0.75	1.50
As*) against structural proteins species <i>Phaseolus vulgaris</i>			
x Ag**) of structural protein species <i>Phaseolus vulgaris</i>	4.84	6.05	6.81
x Ag of structural protein species <i>Phaseolus aureus</i>	2.44	3.58	4.88
x Ag of structural protein species Glycine soya	2.00	2.78	3.78
x Ag of structural protein species <i>Pisum sativum</i>	1.69	2.48	3.25
x Ag of structural protein species <i>Lupinus luteus</i>	1.08	1.61	2.00

*) As = antiserum — **) Ag = antigen

den's method previously (see KLOZ 1960a, KLOZ, TURKOVÁ, KLOZOVÁ 1960), but it seems to us that it is a rather elaborate method requiring much antiserum. Still more demanding is the method of HEIDELBERGER and MACPHERSON (1943), despite which, however, the two methods were very useful.

For a quantitative expression of serological similarities of one sample in Boyden's method of "complete curves", a complete precipitation curve is necessary (the rabbit antiserum is always mixed with antigen diluted in geometric series). Graph 1 (curve 1) gives an example of such a curve. An inhibitory reaction occurs on the left side of the curve where there is a relative excess of antigen. The reaction curve then rises to the point of optimal proportions and falls again until it fades out at very low concentrations of antigens. In this method, the intensity of the reaction is expressed quantitatively by the size of the area under the curve (expressed as a percentage in relation to the homologous reaction which is taken as a 100%). The advantage of this type of evaluation is that it is not necessary to make a precise determination of the original concentration of antigen which is further diluted in geometric series (at lower original concentrations, the curve only moves to the left, at higher concentrations to the right; otherwise, the shape of the curve remains more or less the same). For an objective evaluation of reaction intensity, Boyden uses a horizontally installed Libby's photoreflectometer.

Thus Boyden's method was used as a standard for measuring the QRP method. Serological similarity was determined with four plant species in each case, both with antisera against reserve or structural proteins of the variety *Phaseolus vulgaris* L. (tab. 4 and 5) and with antisera against reserve proteins of the species *Vicia faba* L. (tab. 6). Each series of determinations was carried out with different antiserum (the numbered antisera were of varied titre, avidity and concentration and thus also of different differentiating power). Thus, the conditions for comparison were most unfavourable. The antigens were also prepared for each determination separately and if necessary, even from new preparations.

Table 4. Comparison of results obtained with Boyden's method and quantitative ring precipitation method. Details in text

Intensity of reactions						
	Antisera against reserve proteins of seeds <i>Phaseolus vulgaris</i>					
	Boyden's method	QRP				
Tested reserve proteins species	As* No. 1	As No. 2	As No. 3	As No. 4	As No. 5	As No. 6
<i>Phaseolus vulgaris</i>	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
<i>Phaseolus coccineus</i>	88.2	86.4	86.8	88.6	89.4	84.9
<i>Glycine soya</i>	3.4	0.4	4.6	0	0	3.4
<i>Vicia faba</i>	1.8	0	2.0	0	0	0

The results obtained by using antisera against reserve proteins of the species *Phaseolus vulgaris* L. are given in tab. 4. The reserve proteins of the seeds of four species were tested by the aid of these antisera, i.e. *Phaseolus vulgaris* L., *Phaseolus coccineus* L., *Glycine soya* (L.) Sieb. Zucc. a *Vicia faba* L. In spite of the fact that antisera of varied characteristics were used, there is good agreement between the results obtained by the method QRP and Boyden's method.

The results obtained using antisera against structural proteins from the subcotyledoneous parts of seedlings (hypocotyl + root) of the same species as in table 4, are given in table 5. The unequal differentiating power of different antisera in both methods is here much more marked. Otherwise, there is no substantial difference in the proportionality of the results and neither, of course, in the sequence of the species.

Table 5. Comparison of results obtained with Boyden's method and quantitative ring precipitation method. Details in text

Intensity of reactions					
	Antisera against structural proteins <i>Phaseolus vulgaris</i>				
	Boyden's method		QRP		
	As* No. 1	As No. 2	As No. 3	As No. 4	As No. 5
<i>Phaseolus vulgaris</i>	100.0%	100.0%	100.0%	100.0%	100.0%
<i>Phaseolus coccineus</i>	94.4	76.9	97.4	93.4	97.2
<i>Glycine soya</i>	63.0	25.9	29.3	40.1	42.4
<i>Vicia faba</i>	29.0	11.4	16.6	18.2	24.8

* As = antiserum

Table 6. Comparison of results obtained with Boyden's method and quantitative ring precipitation method. Details in text

Tested reserve proteins species	Intensity of reactions	
	Antisera against reserve proteins of seeds <i>Vicia faba</i>	
	Boyden's method	QRP
	As No. 1	As No. 2
<i>Vicia faba</i>	100.0%	100.0%
<i>Glycine soya</i>	4.6	9.8
<i>Phaseolus coccineus</i>	0.8	0.1
<i>Phaseolus vulgaris</i>	0	0

As = antiserum

Tab. 6 gives the results which were obtained when antisera against reserve proteins of seed from the species *Vicia faba* L., *Vicia faba* L., *Glycine soya* L. SIEB. ZUCC., *Phaseolus coccineus* L., *Phaseolus vulgaris* L., were again tested with this antiserum. QRP was carried out with one antiserum and Boyden's "complete curve" with the other. The agreement between the two methods is again very good.

Otherwise, both the preparation of antisera and the carrying out of the reactions were the same in these cases as in the previous experiments.

Discussion and Conclusions

In experiment No. 1, two antisera of varied characteristics were used as they normally occur in experimental practice. From graphs 2, 3, 4 and 5 it is clear that the differentiating power of antisera with a low titre and avidity can be substantially increased by raising the antiserum concentration to 28% (while a 7% concentration of protein in the serum is considered normal). It is also possible to raise the differentiating power by raising the concentration of antigen but only up to the limit of about 1 mg. in 1 ml., or possibly 1.5 mg. in 1 ml. If a higher concentration of antigen is used, it is better to make a trial curve before the actual experiment, (i.e. at least several reactions of the antiserum used with a gradually increased concentration of antigen) to test whether the use of a higher antigen concentration does not invoke an inhibition reaction as is the case, for instance, in graph 2 with 7% antiserum. In addition to this, the use of a higher antigen concentration (approx. over 1 mg./1 ml.) could invoke interference from the possible and quite frequent presence of non-specific precipitins in the tissues of the plant. They are usually the known ones such as phytohaemagglutinins, however, besides haemagglutination, precipitation of serum components also occurs. Otherwise, as in further experiments, the objectivity of the results obtained through QRP are obvious from the regularity of the graph.

It was ascertained in experiment No. 2 that no anomalies occur when using polyvalent antisera with different concentrations of antigens. Antiserum against the whole complex of structural antigens of the species *Phaseolus vulgaris* L. were used (tab. 3 and graph 6). Only the differentiating power of the system changes regularly.

It is obvious however, that when determining the intensity of cross reactions by the use of QRP, a precise determination of the amount of protein (antigen) is necessary together with the dilution to exactly similar concentrations of the tested solutions.

There is one more difference between Boyden's method and QRP. Boyden's method of "complete curves", for instance, allows the differentiation of the quantitative changes in the antigen from the qualitative changes when using one antigen and monovalent antisera (see Boyden 1954). The Boyden method, in this case, detects only qualitative differences in the specificity of a certain antigen. If, on the other hand, the differences are only quantitative (in the amount of a certain antigen in the mixture) then Boyden's method gives practically the same curve which moves, for instance, only relatively towards the left in the case of a smaller amount of tested antigen (towards a higher total concentration of the whole mixture of antigens—see Boyden's method evaluation on graph 1 curve 1).

When investigating antigens in mixtures by the QRP method, however (i.e. reactions with one standard concentration of antigen mixture), quantitative differences are not distinguished from qualitative, for example, in investigating a certain antigen. The reason for this is that quantitative changes always go inseparably hand in hand in such a case with qualitative changes. It is of no consequence whether there is for instance only half the amount of antigen in the heterologous reaction in comparison with the homologous reaction, or if the same amount (as against the homologous reaction) is present but with only half similarity in specificity (i.e. for instance, with only 50% determination groups reacting in conformity): in both cases the same amount of antigen of different specificity remains up to 100%. These quantitative differences in antigens in mixtures are always accompanied by qualitative differences (with taxonomical significance in comparative serology) in the QRP method.

When comparing Boyden's method with QRP, on tab. 4, 5, 6, (experiment No. 3) it follows that QRP has no disadvantages as far as accuracy is concerned. The differences are caused by methodical errors (which in both methods are about $\pm 3\%$) but mainly by the dissimilar differentiating power of different antisera.

Otherwise, the QRP method has the advantage of being extremely economical in its requirement of antisera (0.05 ml. antisera is needed for one determination as compared with 2.0—3.0 ml. for the method of "complete curves"). It is also economical in the use of working time (instead of 10 reactions for one determination, only one reaction is necessary). A certain disadvantage, however, is the necessity for the accurate determination of antigen concentration. In other respects, both methods have equal requirements in relation to cleanliness and accuracy. The reproducibility is very good in the QRP method (see KLOZ 1960b).

QRP is suitable for the purposes of quantitative work in immunochemistry and for comparative serology.

Acknowledgement

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Использование количественной кольцевой преципитационной реакции для определения перекрестных реакций

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В работе приводятся практические указания и примеры использования количественной кольцевой преципитации для определения интенсивности перекрестных реакций. Проверилась способность этого метода объективно воспроизводить интенсивность реагирующих систем антиген — антитело в условиях разных концентраций. Проведено сравнение метода Бойдена (метода «полных кривых») и количественной кольцевой преципитации.

Выгодой использованной количественной кольцевой преципитации является экономия антисыворотки (для одного определения хватит 0,05 мл антисыворотки), меньшая затрата рабочего времени и, повидимому, меньшая чувствительность по отношению к неспецифическим факторам.

Количественная кольцевая преципитация (ККП) является подходящим методом для количественных экспериментов в иммунохимии и для целей сравнительной серологии.

Wheat Dwarf Virus Disease

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Virová zakrslost pšenice

V práci je podán důkaz o virové povaze zakrslosti pšenice, vyskytující se v některých oblastech ČSSR. Virus se podařilo přenést přirozeně infikovanými imagy kříška *Psammotettix alienus* DAHLB. na jarní pšenici a jarní ječmen. Rovněž se ukázalo, že je ve vysokém procentu přenosný larvami vypěstovanými z vajec a uměle infikovanými na zdroji nákazy. Pokus o přenos viru mšicemi *Rhopalosiphon oxycanthae* (SCHRANK.) a *Sitobium granarium* (KIRBY) MORDV. byl negativní. Rovněž se nepodařilo přenést virus půdou, obilkami a mechanickou inokulací šťávy z nemocných rostlin.

Virus zakrslosti pšenice by mohl podle přenašeče stát nejbližší viru ruské mozaiky ozimé pšenice. Liší se však od něho symptomy vyvolávanými na hostitelských obilovinách, jiným vektorem a nedostatkem inklusí v cytoplasmě infikovaných rostlin. Tyto skutečnosti naznačují, že jde o nový doposud nepopsaný virus.

Summary

Proof is given in this paper that dwarfing of wheat, which occurs in several regions of Czechoslovakia, is a virus infection. The virus was transmitted by means of naturally infected imagos of the leaf-hopper *Psammotettix alienus* DAHLB. to spring wheat and spring barley. It was also found that it is transmissible to a high degree by larvae grown from eggs which are then artificially infected at the source of infection. An attempt to transmit the virus by means of the aphids *Rhopalosiphon oxycanthae* (SCHRANK.) and *Sitobium granarium* (KIRBY) MORDV. was negative. Nor was it possible to transmit it by soil, wheat grains or by mechanical inoculation with sap from diseased plants.

The Russian mosaic virus of winter wheat would seem, in view of its vector, to be the most closely related to the wheat dwarf virus. It differs, however

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as regards the symptoms produced on the host cereals, by having a different vector and by the absence of inclusions in the cytoplasm of infected plants. These facts indicate that we are probably dealing with a new virus, which has not previously been described.

Introduction

In 1960 winter wheat suffered considerable damage in certain parts of Moravia (Haná, southern Moravia) and Bohemia (the vicinity of Prague). Attention was drawn to this by DLABOLA (1960), who pointed to the marked occurrence of the leaf-hopper *Psammotettix alienus* DAHLB. on the diseased crops and, according to his later personal communication, this insect is the cause of the disease. In the light of previous experience with virus diseases of cereals in Czechoslovakia it could be assumed that in this case, too, virus infection was involved. A general investigation of this question has been made during the vegetation period of 1960.

Material and Methods

Imagos and larvae of the leaf-hopper *Psammotettix alienus* were used for the vector experiments. The imagos were naturally infected and were taken from diseased wheat crops in the Ruzyně and Jesenice localities near Praha. Larvae were reared under controlled conditions in a glasshouse from eggs that had been inserted into wheat plants. Four to six days after hatching some of the larvae were put on artificially diseased wheat for acquisition feeding. Following a period of fifteen days on the source of infection they were used for the infection experiments. The other group of larvae, which was not brought into contact with diseased wheat, was used as a standard as regards the effect of feeding on the plant. The imagos and both groups of larvae were then placed on healthy seedlings of the cereals for individual test feeding. Test feeding lasted 6–8 days in the individual passages. Silo cages, as already described (PRŮŠA, JERMOLJEV and VACKE 1959), were employed in order to prevent the insects from escaping.

In experiments on the transmission of the disease by means of aphids the species *Rhopalosiphon oxycanthae* (SCHRANK) and *Sitobium granarium* (KIRBY) Mordv. were used. Following two weeks' feeding on the diseased wheat, the aphids were transferred in groups of 5 to 10 to isolated healthy plants in silo cages. After 7 days the cages were removed from the plants and the aphids were killed with 0.05% solution of Systox. Forty two wheat plants were tested with *Rhopalosiphon oxycanthae* and 48 with *Sitobium granarium*.

Transmission of the virus by soil was investigated in an experiment in which grains from healthy plants were planted in 20 pots which had been filled with soil taken from the immediate vicinity of diseased wheat in the field. After the seedlings had sprouted, 10 were left in each flower pot and were observed up to the time of earing. The standard consisted of plants grown in soil taken from a field with root crops.

In order to examine the transmissibility of the virus by seeds, grains of diseased spring wheat var. Ruzyňská II were used. They were sown in wooden boxes containing soil which had been sterilised by steam. Out of 453 seeds sown, 304 seedlings sprouted and were observed up to the time of earing.

In the experiments on mechanical transmission sap expressed from diseased wheat plants was used. The preparation of the inoculum and the method of inoculation were similar to those used in the investigation of mechanical transmission of the wheat striate virus (PRŮŠA and VACKE 1960). A total of 112 wheat plants and 45 barley plants were inoculated.

The material tested in the infection experiments consisted of spring wheat var. Ruzyňská II and spring barley var. Stupický plnozrnný at the vegetative stage of 1–2 leaves. In order to

exclude the possibility of any other infection, the experimental plants were placed in silon isolators.

Diseased plants of wheat and barley, both naturally and artificially infected, were subjected to cytological examination. The method used for this purpose was that which gave such remarkable results in the U.S.S.R. during the examination of plants suffering from the "zakukli-vaniye" virus of cereals and the Russian mosaic virus of winter wheat (SUKHOV 1940, 1942, GOLDIN 1954).

Results and Discussion

Both natural and artificial infection by the leafhopper *Psammotettix alienus* gave rise to symptoms in spring wheat and spring barley identical with those in diseased plants of these cereals under field conditions. With insects caught at Ruzyně transmission was successful in a relatively high proportion of cases (Table 1). The percentage transmission fluctuated according to the different periods of test feeding. Two leafhoppers completely failed to transmit the disease (one died at the fourth, the other at the fifth passage). Out of six insects caught at Jesenice near Prague, only one caused infection. This difference in the infectivity of the vectors from the two localities may have been due to the fact that the extent of the disease differed in these places. At Ruzyně the insects were caught on wheat crops in which the proportion of dwarfed plants was higher than 50%, while at Jesenice diseased plants occurred as individuals at the edges of the field.

In experiments with leafhopper larvae infected on diseased wheat plants a remarkably high proportion of positive transmissions was obtained (Table 1). In the first test with 56 plants, 46 became diseased (82.1%), in the second out of 44 plants 32 were diseased (72.7%). Plants on which non-infected

Table 1. Results of Experiments with the Leafhopper *Psammotettix alienus*

Species of test plant		Wheat				Barley				Wheat	
Test feeding		I		II		III		IV		V	
		Plants		Plants		Plants		Plants		Plants	
		fed on	infected	fed on	infected	fed on	infected	fed on	infected	fed on	infected
Imagos from	Ruzyně	9	5	7	3	6	4	5	4	2	1
	Jesenice	6	1	6	1	5	1	—	—	—	—
Artificially infected larvae		56	46	44	32	—	—	—	—	—	—
Non-infected larvae		31	—	28	—	—	—	—	—	—	—



Fig. 1. Wheat infected with wheat dwarf virus; left a healthy plant. (Photo M. Novák.)



Fig. 2. Spots on wheat leaves affected by wheat dwarf virus disease; left, leaf from a healthy plant. (Photo M. Novák.)

larvae and non-infected adults of *Psammotettix alienus* were put to feed showed only a temporary retardation of growth. This provided proof of the virus nature of the disease under investigation.

The incubation period for the wheat dwarf virus was 14–15 days on the average for both wheat and barley under glasshouse conditions. It fluctuated, however, within the limits of 10 to 25 days.

The attempt to transmit wheat dwarf virus by means of the aphids *Rhopalosiphon oxycanthae* and *Sitobium granarium* was not successful. Experiments to transmit the virus by soil grains of wheat and by mechanical inoculation with sap from diseased plants also gave negative results.

Simultaneously with the etiological study of the wheat and barley disease, symptomatological observation was carried out on both naturally and artificially infected plants.

The most important symptom of wheat dwarf virus disease in the cereals examined is the marked dwarfing (Fig. 1); plants infected at the stage of 1–2 leaves, under glasshouse conditions, attained a height of only 10–15 cm., while the standard plants were over one metre (measured after the earing of healthy plants). Tillering was repressed in wheat and barley infected in the glasshouse. On the other hand, plants infected in the field showed a somewhat greater number of tillers than the healthy plants.

The ears of diseased plants under field conditions were very frequently barren, in many cases they did even emerge from the sheath. In so far as grains were formed in the ears, they were shrivelled.

The retardation of growth caused by the wheat dwarf virus was accompanied by colour symptoms. Fine spots and patches with indistinct outlines were irregularly scattered over the leaves (Fig. 2). At a more advanced stage of the disease the leaves went yellow, starting from the tips and margins. This yellowing later spread to the leaf sheath and stalk. At the final stage of the disease the plants began to go brown and to die off.

The cereal virus that has been determined during this investigation could, in view of its vector, be related to the Russian mosaic virus of winter wheat as known in the U.S.S.R. (ZAZHURILLO and SITNIKOWA 1939). It is, however, very probable that two viruses are not identical, for they differ from each other in many respects. The vector of the Russian mosaic virus of winter wheat (*Psammotettix striatus* L.) is of the same genus as the vector of our virus, but a different species. The differences in the symptoms produced in the host plants may be regarded as being of greater significance.

Lemon-yellow, sharply defined, conspicuous spots and stripes running parallel to the leaf veins develop on the leaves of wheat plants attacked by the Russian mosaic virus of winter wheat (SUKHOV and RAZVYAZKINA 1955). In the case of the present virus, however, only indistinctly outlined, blurred spots are to be found scattered over the whole leaf blade. Their colour varies from light green, dirty yellow to light brown.

As distinct from the Russian mosaic virus of winter wheat, which strongly stimulates tillering, the present virus either does not stimulate tillering at all, or only very slightly.

The fact that under the influence of acid fixation agents the crystals characteristic for the Russian mosaic virus of winter wheat (SUKHOV 1940, GOLDIN 1954) were not formed in the cells of chlorotic tissues of dwarfed wheat also points to a lack of identity between the two viruses. Not even by the preparation of herbarised material was it possible to produce crystal formation. DLABOLA (personal communication) also failed to find crystals in the alimentary tract of leafhoppers from the areas where the occurrence of the disease was most pronounced.

Finally, the behaviour of the viruses in their vectors is not similar. The larvae of *Psammotettix alienus* acquired a high ability of infecting healthy plants with the wheat dwarf virus by feeding on the source of infection. Larvae of *Psammotettix striatus*, however, only rarely transmit the Russian mosaic of winter wheat (GORLENKO 1951).

The facts that have been established indicate that we are probably dealing with a new virus which has not been previously described. It has been named wheat dwarf virus according to the most characteristic symptoms and the first plant identified as its host.

Acknowledgement

The author's thanks are due to Dr. A. Pintera of the Biological Institute of the Czechoslovak Academy of Sciences for kindly determining the aphids and to Dr. J. Dlabola of the Research Institute of Plant Production, Czechoslovak Academy of Agricultural Sciences, Ruzyně for identification of leafhoppers.

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Вирусная карликовость пшеницы

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В работе приводится доказательство вирусной природы карликовости пшеницы, распространенной в некоторых областях ЧССР. Вирус удалось перенести естественно инфицированными имаго цикадки *Psammotettix alienus* Dahlb. на яровую пшеницу и яровой ячмень. Оказалось также, что он в высоком проценте передается личинками вылупившимися из яиц и искусственно инфицированными на источнике заражения. Опыт переноса вируса тлями *Rhopalosiphon oxycanthae* Schrank. и *Sitobium granarium* (Kirby) Mordv. дал негативные результаты. Также не удалось перенести вирус почвой, семенами и механической инокуляцией сока из больных растений.

Вирус карликовости пшеницы по своему переносчику может стоять ближе всего к вирусу русской мозаики озимой пшеницы. Однако, отличается от него симптомами, вызываемыми на злаках-хозяевах, другим переносчиком и отсутствием включений в цитоплазме инфицированных растений. Эти данные свидетельствуют о том, что мы имеем дело с новым, пока не описанным вирусом.

On the Determination of the Course and Termination of Photoperiodic Sensitivity in Spring Wheat (*Triticum aestivum* L.)

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K stanovení průběhu a konce fotoperiodické citlivosti u jarní pšenice (*Triticum aestivum* L.)

Ve fotoperiodických pokusech s jarní pšenicí Niva jsme sledovali průběh fotoperiodické citlivosti a umístění období fotoperiodické reakce v ontogenesi rostlin. Nepodařilo se nám u této dlouhodobní rostliny najít takové období, během něhož by zkrácený den vůbec neměl vliv na rychlost vývoje. Některé údaje však naznačují, že můžeme vymezit období zvýšené fotoperiodické citlivosti, které by odpovídalo období fotoperiodické reakce u krátkodenních rostlin. Výsledky nasvědčují rovněž tomu, že toto období nekončí náhle, nýbrž postupně přechází v následující období, kdy délka dne působí na rychlost vývoje již jen prostřednictvím fotosynthesy. Tento vliv je dobře patrný při použití takových indikátorů jako je vývoj vzrostopného vrcholu a metání. Existenci přechodného období na konci období zvýšené fotoperiodické citlivosti a jeho souvislosti s fází vzrostopného vrcholu od zakládání klásku do zakládání tyčinek je třeba ověřit dlouhodobým pokusem v přísně regulovatelných podmínkách.

Z metodik sledování průběhu fotoperiodické citlivosti se u našeho pokusného materiálu nejlépe osvědčilo metání, které poskytlo křivky s určitými, více nebo méně zřetelnými zlomy, a také sledování abnormit (při klasickém uspořádání pokusu), které indikují narušení vztahu mezi růstem a vývojem. Orientační údaje poskytlo rovněž měření délky rostlin u klasického uspořádání pokusu. Nejméně spolehlivé byly v našich pokusech analýsy vývojového stavu vzrostopného vrcholu.

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Summary

Photoperiodic experiments with the spring wheat Niva were undertaken to investigate the course of photoperiodic sensitivity and localization of the photoperiodic reaction in the ontogeny of plants. The long-day plant used, however, was not amenable to detecting a period during which a shortened day would be completely devoid of effect on the rate of development. Some data indicate, however, that a period of increased photoperiodic sensitivity can be defined which would correspond to the period of photoperiodic reaction of short-day plants. The results also seem to indicate that this period does not terminate abruptly but gradually passes over into the ensuing period when the day length affects the rate of development solely through photosynthesis. This effect is well marked when such indicators as the shoot apex development and earing are employed. The existence of this transition period at the end of the period of increased photoperiodic sensitivity and its connection with the shoot apex phase from initiation of spikelets to initiation of stamens must be verified by a long-term experiment under strictly controlled conditions.

Among the criteria used to examine the course of photoperiodic sensitivity earing appeared to be most suitable as it yielded curves with definite, more or less marked breaks; the investigation of abnormalities (in a classical arrangement), too, proved to be of value, as they indicate interference with the relationship between growth and development. Orientation data were also obtained by measuring plant size in a classical arrangement of the experiment. Analyses of the developmental phase of the shoot apex proved to be of least value in the experiments described here.

Introduction

The problem of the time position of the period of photoperiodic reaction in plant ontogeny and of its course is associated with the basic problems of the formulation of the developmental theory. One of the basic principles of the theory of stage development is the pronounced change in plant requirements as to ecological factors during ontogeny (LYSENKO 1949). The application of the photoperiodic reaction in studying the change of requirements possesses the advantage that this developmental reaction proceeds directly from the beginnings of generative differentiation of the shoot apex. However, its investigation presents technical difficulties in long-day plants. The assumption concerning the definition of the light stage according to the termination of the day-length reaction, based on work carried out with short-day plants (HOŘAVKA et al., see GORZHAVKA 1956) was shown not to be applicable to long-day plants (BLUM and KRZHIZH 1956). The short day influenced the development of spring wheat up to earing.

In isolated cases it was possible to determine in a similar manner the end of photoperiodic sensitivity even in long-day cereals (WORT 1941, BAS-

SARSKAYA 1946, NOVIKOV 1953, SĂLĂGEANU et al. 1954). The first two of the above authors determined the length of the photoperiodic reaction during uninterrupted illumination. The next two papers deal with the natural day length, but the first of them represents a very rough experiment and the other describes experiments with rye. Most authors failed in finding a period during which the wheat would not react to a shortened day (EREMENKO 1939, OLENIKOVA 1946, RAZUMOV 1948, GRIES et al. 1956, RIDDELL et al. 1958, PETR 1960). It is for this reason that some authors thought that the period which marked the end of the light stage was that corresponding to the greatest difference in the developmental reaction to a shortened day. The boundary was represented either by the difference between variants earing and those not earing in the course of the experiment (MIROSHNIKOV 1953), or between variants with a markedly and minutely inhibited development of the shoot apex (FEDOROV 1953, PETR 1960), or finally by the greatest leap of the curve of earing of the individual variants of the photoperiodic experiment (PETR 1960).

Most authors dealing with the time limitation of the light stage with the aid of photoperiodic sensitivity attempted to determine this stage, particularly its end, according to a certain developmental stage of the shoot apex. Opinions concerning this indication vary. According to STANKOV (1938) and SAPEGIN (1939, 1940) the light stage ends before the formation of sporogenic tissue. According to EREMENKO (1939) the differentiation of the shoot apex takes place during the light stage. ZABLUDA (1939) and NOVIKOV (1953). SKVORTSOV (1956) and KUPERMAN (1959) placed the end of the light period at the beginning of initiation of stamens in florets; finally according to BASSARSKAYA (1946), OLENIKOVA (1946), FEDOROV (1953, 1954), PETR (1960) and to earlier work of KUPERMAN et al. (1955) the light stage ends even before initiation of spikelets. PAVLOV (1956) divided the light stage into two periods: 1. preceding spikelet differentiation, 2. following spikelet differentiation. RIDDELL et al. (1958) also found that the highest photoperiodic sensitivity corresponds to the developmental phase preceding spikelet differentiation.

RAZUMOV (1948) takes up the formation of abnormalities after transfer to a short-day régime. According to him, the length of the light stage is determined by the shortest long-day treatment which will still ensure the development of morphologically typical, non-proliferating inflorescences. Our results are also in agreement with his (SEIDLOVÁ-BLUMOVÁ 1961).

The present work was devoted to the investigation of photoperiodic sensitivity of a spring-wheat sort as a representative of the group of long-day plants. During the experiments different methods of indicating the course of the photoperiodic reaction were tested.

Methods

Photoperiodic experiments were carried out in the years 1956—1959 with the spring wheat Niva. The so-called "gradual" technique of photoperiodic experiment was used here (GORZHAVKA et al. 1956). Experimental variants remained under the short-day conditions for 12 days. The interval between the beginning of treatment in adjacent variants amounted to 3 days. The

short-day was generally an eight-hour one, in 1959 also a ten- and twelve-hour one. All the technical details and the time of the beginning of experiments in the individual years corresponds with those given earlier (SEIDLÖVÁ-BLUMOVÁ 1961). The results of these experiments can thus be compared with those obtained in a classical arrangement of the photoperiodic experiment described in the previous paper.

A number of indicators of the rate of development were compared. Differences in the beginning of earing (appearance of at least one spikelet), in the full earing (appearance of all spikelets), in the development of the shoot apex, in the length of the shoot apex and in the height of plants were investigated. The occurrence of abnormalities was also applied as indicator of the day length effect. The results shown graphically in Figs. 1—9 refer to the age of plants (in days) prior to the treatment and to the developmental stage of the shoot apex prior to the treatment. Meteorological observations made during the experiment indicated a great variety of weather conditions during the individual repetitions of experiments.

Results

The results shown graphically in Figs. 1—3A indicate that even during a short twelve-day photoperiodic treatment the photoperiodic sensitivity is displayed for the whole duration of ontogeny up to earing. There was a certain period, however, when the earing was delayed more, viz. when the treatment began:

in	at the age of	during the shoot apex phase
1956	4—27 days	1—5
1957	10—28 days	1—5
1959	11—28 days	1—5

In common with this, all the variants of the "classical" experiment reacted to the shortened day (Fig. 4—7A). In most cases, however, a part of the curve could be defined which corresponded to a stronger reaction to day length. A steeper section of the curve,

Fig. 1—3. Results obtained during the "gradual" arrangement of the photoperiodic experiment in different years.

Fig. 4—9. Results obtained during a "classical" arrangement of the photoperiodic experiment in different years. On the abscissa — age of plants in days (from appearance above ground) at the beginning of the photoperiodic experiment. The onset of individual phases of the shoot apex (according to the previously used scale (SEIDLÖVÁ-BLUMOVÁ 1961) is shown below. A — The course of earing (-----) and completion of earing (——) of variants with different beginning of treatment compared with the control (horizontal line). On the ordinate — age of plants in days at time of taking to ear. B — Stage of development of the shoot apex with simultaneous analysis of all variants with a different beginning of photoperiodic treatment (at the right, plant age at the time of analysis). The ordinate shows the phases of shoot apex development. C — The height of plants after reaching maturity in variants with a different beginning of photoperiodic treatment as compared with the control (horizontal line). D — Occurrence of plants with supporting bracts in spikes following a different beginning of photoperiodic treatment.

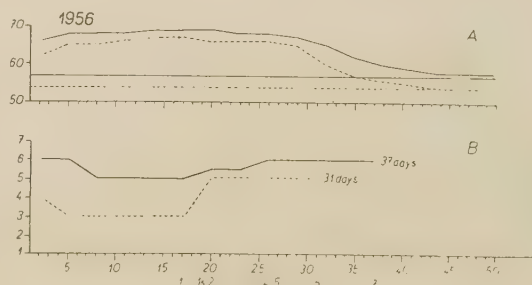


Fig 1

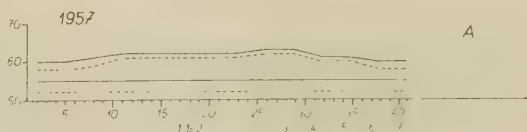


Fig 2

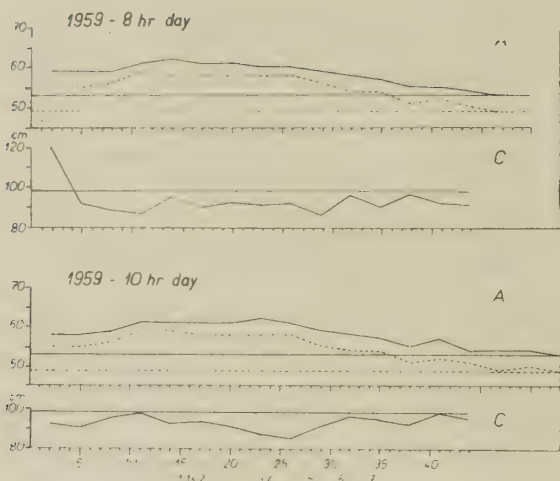


Fig 3a,b

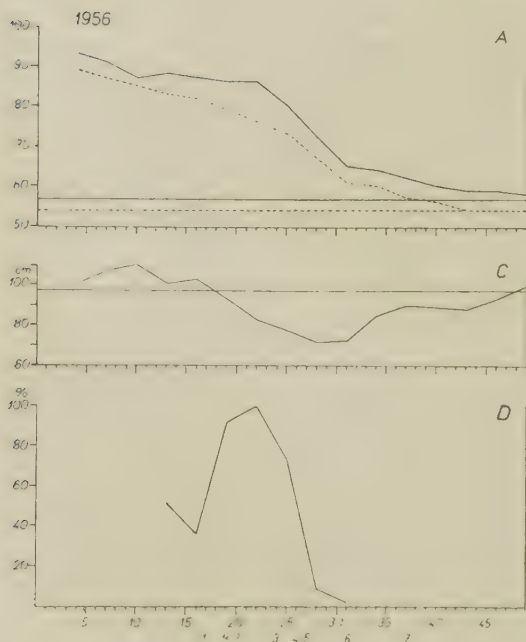


Fig 4

which could correspond to the period of transition between the stronger and weaker reaction to day length, usually represents not only a difference between two adjacent variants but comprises several variants. This transition appeared:

in	at the age of	during the shoot apex phase
1956	22—31 days	2—6
1957	22—34 days	2—5
1958	23—24 days	2—3
1959	20—23 days	2—3

More marked differences in the curves of earing are caused by abnormal ears with bracts.

In 1956 and 1959, parallel „classical” experiments were carried out in which the short day displayed an influence up to the time when the natural day length decreases rapidly (up to August 20). The boundary between variants earing and those not earing was examined. In these experiments the only variants not earing were those transferred to a short-day régime during a very early developmental stage. The different developmental stage of the shoot apices revealed during analysis of these variants (not earing) that the boundary between the variants earing and not earing was decided by the time of termination of the experiment (or shortening of the natural day length toward the end of the vegetation period).

A comparison of the development of shoot apices during the “gradual” method of photoperiodic experiment was carried out in 1956. It follows from

Fig. 1B that the boundary between the delayed variants and the not delayed ones is mobile. When analysis is carried out later the percentage of delayed variants rises. The same holds for analyses of the shoot apex carried out by the “classical” method of experiment.

The differences in plant height were obliterated after ear emergence in the "gradual" method (Fig. 3C). The differences in height in the "classical" method (Fig. 4—6C) are related to the formation of abnormalities. Plants with imperfectly developed bracts are always lower than the control. Figs. 4 to 7D show the curves of occurrence of plants with abnormal bracts in the principal spike. The percentage of spikes with this abnormality decreases after treatment:

in	at an age of	during the shoot apex phase
1956	23—26 days	3—4
1957	28—31 days	3—4
1958	25—30 days	3—5
1959	23—24 days	3—4

In 1959, the same experiment was repeated using a ten-hour and a twelve-hour day (from 6 a.m. to 4 p.m., and from 6 a.m. to 6 p.m., respectively). Not even in these cases was it possible to define the period of insensitivity to day length. The shoot apex development was most rapid with the twelve-hour day and slowest with the eight-hour day. The sequence was maintained with respect to earing of variants with the same beginning of photoperiodic treatment. With the ten-hour and twelve-hour shortened day, all variants took to ear eventually even if they remained under the conditions of a shortened day until the

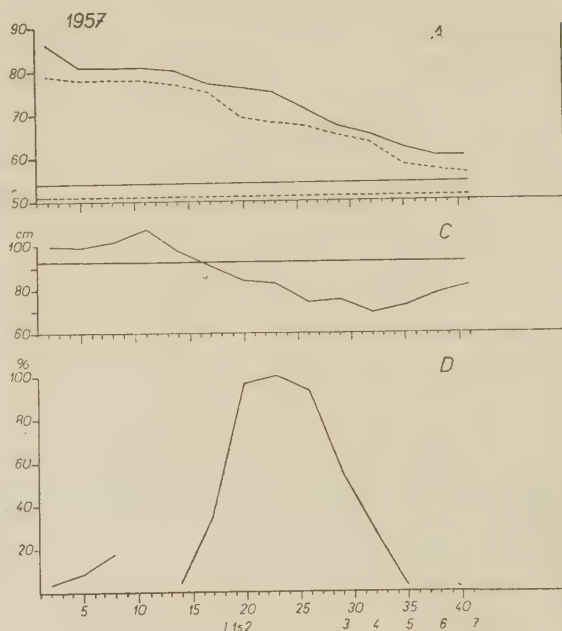


Fig 5

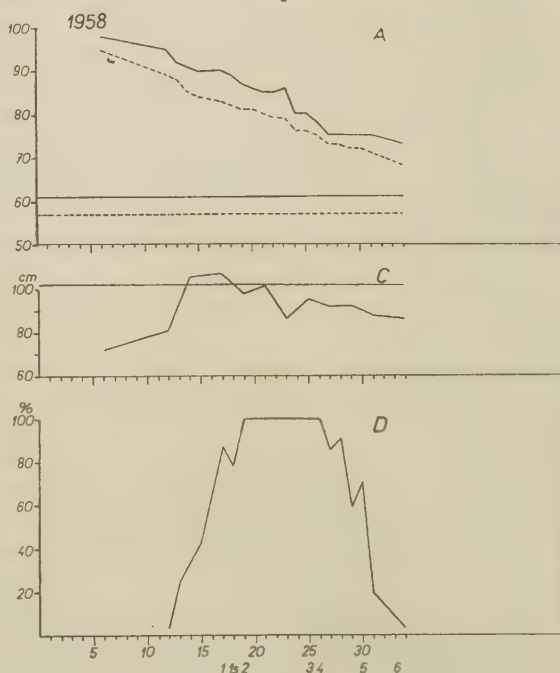


Fig. 6

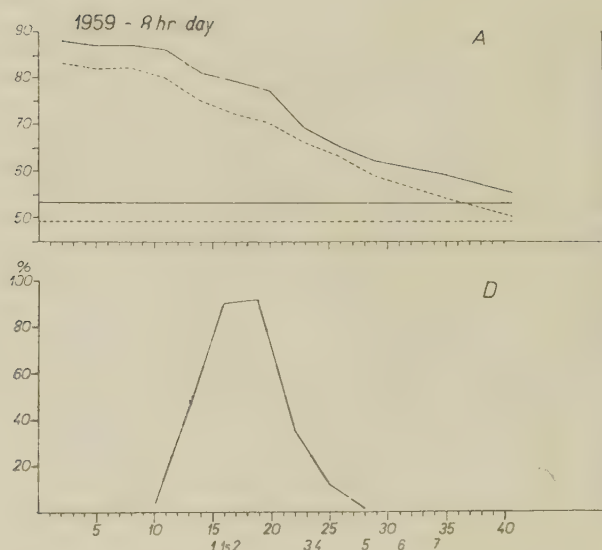


Fig. 7

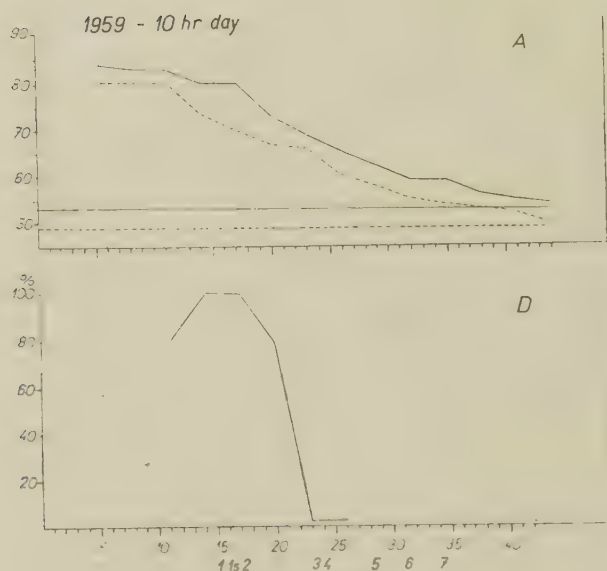


Fig. 8

time of their taking to ear. In spite of this it was shown during the investigation of the course of photoperiodic sensitivity that there exists a difference between the twelve-hour day, on the one hand, and the eight- and ten-hour day, on the other. The course of earing and especially the occurrence of abnormalities have shown that with respect to the spring wheat Niva the twelve-hour day is on the border between the short and the long day.

Discussion

It was quite impossible to determine a period devoid of developmental reaction to day length, both when the "gradual" method with a relatively short photoperiodic treatment was used and when the shortened day was prolonged to 10 or 12 hours. The photoperiodic reaction of short-day plants may be said to be terminated only when the short day exhibits no enhancement of development (HOŘAVKA, see GORZHAVKA et al. 1956). In the long-day plants this specific photoperiodic sensitivity is complicated by a limitation of energy uptake for photosynthesis under the short-day conditions. In studying the shoot apex development and the earing it may be observed

that the morphogenesis of embryonal inflorescence and the growth of stem internodes are slower, apparently partly due to nutrition.

The results of our experiments indicate that the reaction to day length had not been the same all the time preceding the earing. In agreement with OLENIKOVA (1946), RAZUMOV (1948, 1954), FEDOROV (1953), PAVLOV (1956),

GRIES and co-workers (1956) and RIDDELL and co-workers (1958) it was possible to distinguish a period of increased photoperiodic sensitivity in the spring wheat. It was more difficult to define the end of the period exactly. This difficulty might explain the disagreement concerning the relationship of the light stage (defined as the end of photoperiodic sensitivity) to a definite morphogenetic degree of development of the shoot apex. It may be seen

however, that in more recent papers the end of the light stage is either identified with spikelet differentiation (BASSARSAKAYA 1946, OLENIKOVA 1946, FEDOROV 1953, KUPERMAN et al. 1955, PETR 1960) or with stamen differentiation in florets (NOVIKOV 1953, SKVORTSOV 1956, KUPERMAN 1959). This difference in the results corresponds to the range of shoot apex development in which the termination of the period of increased photoperiodic sensitivity was found to lie. No more exact data could be obtained.

The results of the present experiments as well as the analysis of the literature relevant to the topic indicate that the difficulty of an exact definition of the period with increased photoperiodic sensitivity is not due to the technique used alone. It appears quite likely that the increased photoperiodic sensitivity corresponding to the period of photoperiodic reaction of short-day plants decreases gradually and there remains but a non-specific reaction to the shortened period of photosynthesis. Curves of photoperiodic sensitivity of the short-day plant *Perilla crispera* obtained by WELLENSIEK (1958) suggest that the gradual fading out of photoperiodic sensitivity could represent a more general phenomenon. In the spring wheat Niva the transition period could coincide with the time of initiation of spikelets, florets and stamens in florets. Since, however, these results, were obtained in a field experiment under variable weather conditions can be of an orientation value only. The answer to the question of the character of the course of photoperiodic sensitivity can be obtained exclusively by long-term experiments under strictly controlled conditions.

On the basis of several-year experiments the techniques of photoperiodic experiments and their interpretation may be compared. In the present work the technique of comparing development at two day lengths which has been of value in comparing the length of the light stage on a wide scale was not used (DOLGUSHIN 1935, TELTSCHEROVÁ 1955). The method was criticized by PETR (1959). It is especially unsuitable for a more detailed study of a given plant material as it confuses the length of the photoperiodic reaction period with photoperiodic sensitivity during this period. A similar orientation character can be ascribed to the technique of successive sowing used by

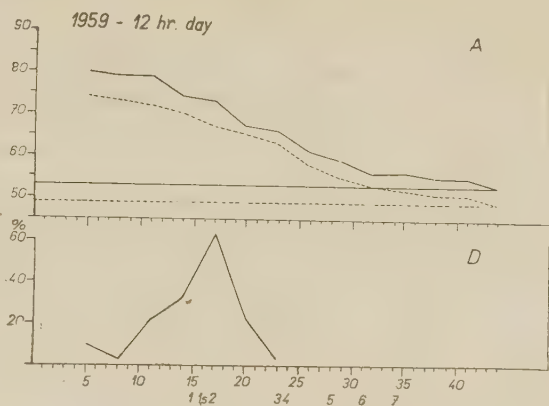


Fig. 9

KUPERMAN and co-workers (1955) and to a combination of both techniques as found in the papers of MATHON (1952, 1953).

For the present work it appeared most useful to divide the photoperiodic experiment into several variants with a different onset of photoperiodic inhibition. The "gradual" arrangement of such a photoperiodic experiment has the advantage not only in being simple and having smaller requirements as to protection against light but also in excluding the factor of the duration of photoperiodic treatment. This is of importance particularly with plants with a less developed photoperiodic reaction where the morphogenesis is slow even under short-day conditions. In the "classical" method there were more marked differences in the development of experimental variants, on the one hand, and abnormalities appeared after a sufficiently prolonged treatment, on the other. According to the occurrence of definite abnormalities an assessment can be made of the course of photoperiodic sensitivity. It was shown in the previous paper (SEIDLOVÁ-BLUMOVÁ 1961) that the last abnormality still to demonstrate that a considerable shift of the relationship between growth and development took place during the short-day régime, is an abnormal development of bracts of the spike. The boundary of increased photoperiodic sensitivity corresponds then to the beginning of photoperiodic treatment in variants with a decreasing percentage of plants exhibiting this abnormality. This is in accord with the results of RAZUMOV (1948, 1954). In the "classical" technique the investigation of development and of abnormalities can be supplemented by measuring the length of plants after reaching maturity. Variants with the beginning of treatment at the end of increased photoperiodic sensitivity displayed, in agreement with PETR (1960), a rather marked decrease in the stem length.

Of all the criteria used the analysis of shoot apices proved the least satisfactory. According to KUPERMAN (1955), HOŘAVKA (see GORZHAVKA 1952), PETR (1959) and other authors the comparison of the developmental stage of shoot apices should provide more exact data than earing. In our case, however, the morphogenesis of ontogenetically more advanced organs proceeded even after shortening the day and only quantitative differences in the shoot apex development, without any marked and steady boundaries occurred between the variants. The shoot apex development can represent a suitable indicator for plants with a very marked photoperiodic reaction, such as for example the alternatives (PETR 1960). A comparison of our results with those of PETR (1959, 1960) obtained with the aid of the same technique and during the same years (i.e. under similar experimental conditions) indicate that for morphological indication of the course of development as well as for the selection of criteria the morphological and ontogenetically ecological characteristics of the plant studied are of importance.

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К установлению течения и конца фотопериодической чувствительности яровой пшеницы (*Triticum aestivum* L.)

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В фотопериодических опытах с яровой пшеницей Нива мы изучали течение фото-периодической чувствительности и изменение периода фотопериодической реакции в онтогенезе растений. Нам не удалось у этого длинодневного растения найти период, в течение которого сокращенный день не влияет на скорость развития. Некоторые данные, однако, свидетельствуют о том, что мы можем определить период повышенной фотопериодической чувствительности, который соответствует периоду фотопериодической реакции у растений короткого дня. Результаты свидетельствуют также о том, что этот период не оканчивается внезапно, а постепенно переходит в дальнейший период, в котором длина дня действует на скорость развития уже только посредством фотосинтеза. Это влияние хорошо заметно при использовании таких индикаторов, какими являются развитие верхушки роста и выколанивание. Существование переходного периода к концу периода повышенной фотопериодической чувствительности и его связь с фазой верхушки роста с момента от закладки колосков до закладки тычинок надо проверить долговременным опытом в строго регулируемых условиях.

Из методик наблюдений течения фотопериодической чувствительности у нашего подопытного материала лучше всего подходит выколанивание, которое дало нам кривые с определенными, более или менее заметными изломами, и также исследование отклонений (при классической схеме опыта), которые указывают на изменение отношений между ростом и развитием. Ориентировочные данные получены также измерениями длины растений в классической схеме опытов. Менее всего надежными оказались в наших опытах анализы развития верхушки роста.

Application of the Leaf-Disk Method to the Determination of Photosynthesis in Cereals

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Použití terčíkové metody ke stanovení fotosynthesy u obilovin

Je popsána úprava terčíkové metody (BARTOŠ, KUBÍN a ŠETLÍK 1960) pro studium intensity fotosynthesy u obilovin. Místo terčíků je použito segmentů 2 cm dlouhých. Z každého listu se razidlem najednou vyseknou dva segmenty, z nichž jeden slouží pro stanovení váhy sušiny na počátku pokusu, druhý se exponuje v komoře za konstantních podmínek. Vzhledem k heterogenitě listu je třeba pro jedno opakování použít alespoň dvou segmentů z opačných částí listu. Je popsána podložka vhodně upravená pro kladení segmentů, aby bylo zajištěno jejich sycení vodou. Jsou uvedeny přírůstky sušiny segmentů z prvních listů rostlin dvou odrůd ozimé pšenice pěstované v pískových kulturách při umělém osvětlení. Přírůstek sušiny je v průběhu 12 hodin lineární a u odrůdy Weibulls Banco dosahuje až 51,5%, což odpovídá přírůstku 122,5 mg/dm².

Summary

A modification of the leaf-disk method of BARTOŠ, KUBÍN and ŠETLÍK (1960) is described for use in studying photosynthesis in cereals. In place of leaf-disks segments of 2 cm. in length are used. Two segments are cut by a punch from each leaf, one segment serving for the determination of dry weight at the beginning of the experiment, the second being exposed in a chamber under constant conditions. In view of the heterogeneity of the leaf, two segments from the opposite parts of the leaf must be used for each sample. A suitably modified support is also described for holding the segments in order to ensure their saturation with water. Dry weight increments of first-leaf segments of two sorts of winter wheat grown in sand cultures

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under artificial illumination are presented. The dry weight increment is linear in the course of 12 hours and with the sort Weibulls Banco it amounts to 51.5% which corresponds to an increment of 122.5 mg./dm.².

The leaf-disk method of estimation of the capacity of photosynthesis based on measuring the dry weight increment (BARTOŠ, KUBÍN, ŠETLÍK 1960) is relatively simple and may be applied to the solution of a number of problems. It does not require complicated apparatus and will certainly be employed for the investigation of numerous economic crops as well as wild-growing plants. For the measurement of photosynthesis in grasses RYCHNOVSKÁ (1959) used planar samples assembled from a greater number of segments of narrow leaves.

Recently, increasing attention has been paid to the investigation of photosynthesis in cereals, which occupy an outstanding position among economic crops. But for these plants the punching out of disks is very inconvenient. During the early stages of growth the cereal leaves are relatively narrow so that the leaf-disks are very small, and the role of peripheral injury is no longer negligible on account of the relatively small surface area of the disk.

During the late growth phases some leaves may reach the width of 2 cm. but their nervature is markedly developed. The leaf-disk dry weight is formed then mainly by the conductive tissue which naturally distorts the true photosynthetic capacity of the assimilatory tissue.

For the above reasons segments were used in this work in place of leaf-disks. Depending on the age and type of leaf it is almost always possible to find the central part of the leaf which has the same width over the length of at least 4 cm. By cutting out two segments of 2 cm length from such a part of the leaf, suitable material for measurement is obtained. One segment is used

as control for dry weight determination at the beginning of experiment, the other is exposed to suitable conditions and after a certain period of time the increment in dry weight (and thus the intensity of photosynthesis) is determined exactly as described in the original method.

For cutting the segments a cutter was devised (Fig. 2 and 4) making it possible to cut out both leaf segments simultaneously, as used by RYCHNOVSKÁ (1959). Three blades (1) held

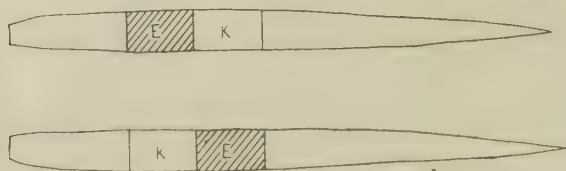


Fig. 1. Removal of segments from two leaves.
K — control, E — experimental.

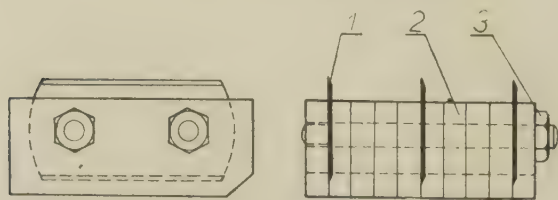
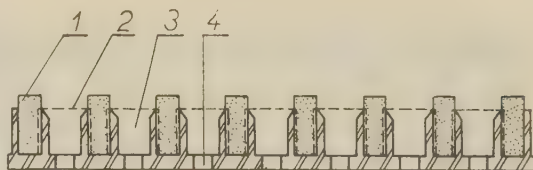


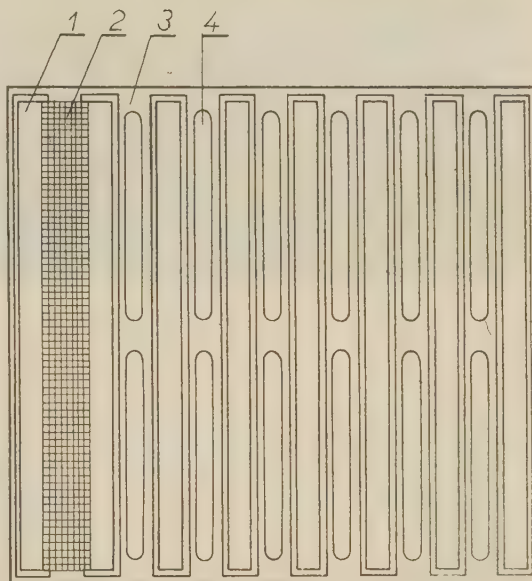
Fig. 2. Cutter for simultaneous cutting of two leaf segments. 1 — blade, 2 — adjustable plexiglass parts, 3 — nut of a screw connecting the adjustable parts.

Fig. 3. Support for segments. 1 — polyurethane foam, 2 — stainless steel mesh, 3 — space ensuring circulation of air below the mesh, 4 — openings in the principal plane of the support intended for easier attachment of segments of the mesh with Silon thread.



between adjustable plexiglass parts (2) can be used. The height of the cutting edge over the plexiglass parts makes it possible to prepare segments from two leaves placed on top of each other simultaneously.

For the exposure of the segments the entire support had to be modified accordingly (Fig. 3 and 5). The segments are now placed on a stainless-steel mesh (2) and with their cutting edges they are in contact with polyurethane foam (1) which is placed in troughs filled with water, the supply of water in these troughs being adequate, for a relatively long time, to provide water for the segments by means of the polyurethane foam. In some cases the leaves are considerably twisted so that segments prepared from them must be attached. For this purpose use was made of a Silon thread which makes it possible to fix the segments at the edge and in the centre of the mesh. Thus it is possible to keep all segments flat with their edges in contact with the polyurethane foam.



The segment dry weight of a leaf section near its apical end is in most leaves lower than that of a section removed from the basal part of the leaf-blade. For this reason, each sample is formed by at least two segments from the opposite parts of the leaf, as shown in Fig. 1. A more powerfully formed conductive system of the basal part is displayed to a different extent in leaves of different species and genera and in leaves of different age and position on the plant. Thus it is necessary to ascertain in advance how many leaves are to be used in order to minimize the differences bet-

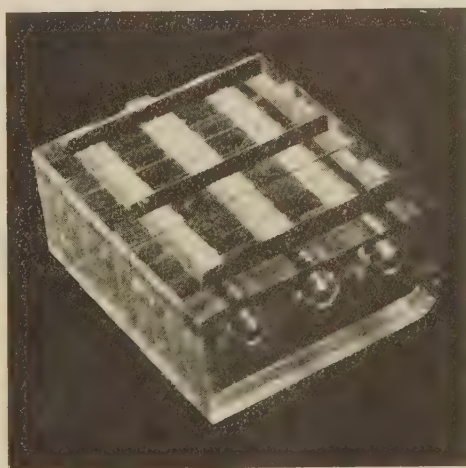


Fig. 4. Cutter for simultaneous cutting of two segments from a leaf.

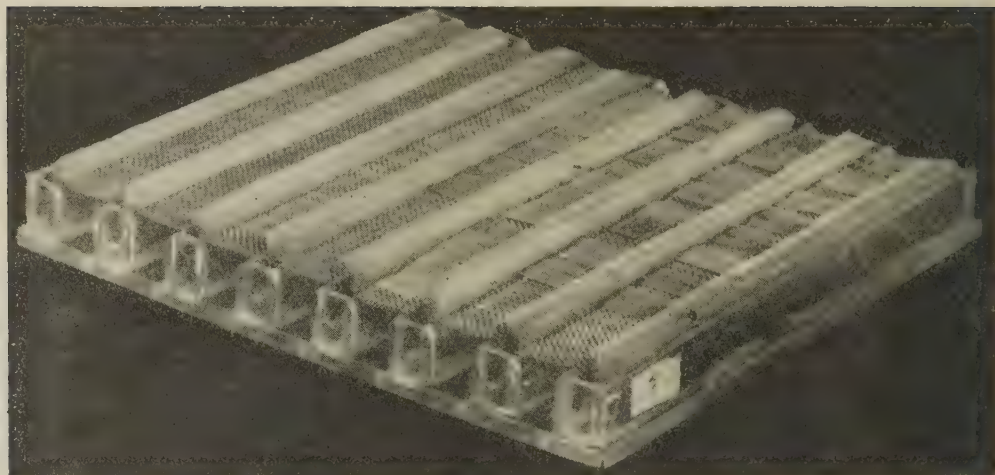


Fig. 5. Support for segments.

ween the two segment sets. For example, with leaves removed from the highest levels of insertion in a field stand, 4 to 8 leaves should be used. This means that for the control and experimental series, 4 segments from the apical and 4 from the basal part of the leaf-blade are removed and in all these 8 segments the dry weight is estimated at once, the intensity of photosynthesis being then referred again to the total area of these 8 segments.

After preliminary tests with different lengths of segments, regular use is now made of the standard length of 2 cm. Shorter segments were not suitable on account of their tendency to twist and of difficulties connected with their saturation with water. With longer segments it is rather difficult to remove two sets of segments, the dry weight difference of which would not exceed 1%. Moreover, the margins of longer segments cannot be kept parallel which rather complicates the determination of the segment area and the calculation of dry weight increments expressed per area unit. For the measurement of area of segments representing a rectangle it is sufficient to measure the width of each segment or the width of all segments placed beside each other and constituting one sample since their length is constant (2 cm.). For the determination of segment width in young plants grown under artificial conditions it is of advantage to use a microscope without eyepiece with the objective Meopta 3. The image of a part of the segment as formed by the objective is projected onto a dull glass. Depending on the distance of this glass from the objective focus it is possible to achieve a 5- to 10-fold linear magnification. The image on the glass is measured with a rule calibrated with the aid of a micrometer objective. This makes it possible to estimate the width of the segment and thus its area with an accuracy of at least 1%. If a sample consists of a greater number of segments the same accuracy is obtained by measuring with an accurate millimeter rule. It was also attempted to measure the width by photographing the support with segments and

Table 1. Determination of dry weight difference between the control and the experimental sets of segments

Two segments used for each determination. K — control, E — experimental

Sample	K	E	Difference $d = E - K$
1	2.162	2.175	+ 0.013
2	2.132	2.160	+ 0.028
3	2.218	2.228	+ 0.010
4	2.692	2.722	+ 0.030
5	2.080	2.095	+ 0.015
6	2.095	2.118	+ 0.023
7	2.500	2.512	+ 0.012
8	3.015	3.033	+ 0.007
9	2.532	2.548	+ 0.016
10	2.658	2.675	+ 0.017
11	3.208	3.182	- 0.026
12	2.615	2.635	+ 0.020
13	2.675	2.662	- 0.013
14	2.225	2.260	+ 0.035
15	2.472	2.450	- 0.022
16	2.788	2.775	- 0.013
17	3.195	3.178	- 0.017
18	3.422	3.410	- 0.012
Mean	2.594	2.605	$d = 0.0068$
Statistical treatment			$s = 0.0141$ $n = 18$ $t = 2.06^*$

*) not significant; for degrees of freedom = 17 and $P = 0.05$, $t = 2.11$.

measuring the negative under the microscope or in a magnifier. This procedure, however, encumbers the measurement and the two first-named ways were found to be completely satisfactory.

The application of a greater number of segments in a single sample has the advantage of decreasing the great fluctuation of measured values as between individual repetitions. This fluctuation may be due to differences in the photosynthetic capacity between individual plants which, according to ŠETLÍK, BARTOŠ and KUBÍN (1960) may amount to as much as 50%.

In spite of this, with young leaves of plants grown in pot cultures it is sufficient to remove two segments in the way shown in Fig. 1. Table 1 shows an example of this in presenting the difference between the control and the experimental set. The segments were removed from plants grown in small plastic pots of 100 ml. capacity. Selected sowing seeds of winter wheat (sort Židlochovická osinatka) were left for 12 hours in moist filter paper and then sowed into washed silica sand (3 seeds in each pot). Distilled water was used for watering. After sowing the pots were illuminated with artificial light (30 fluorescent tubes of 40 W over an area of 300×120 cm., the distance

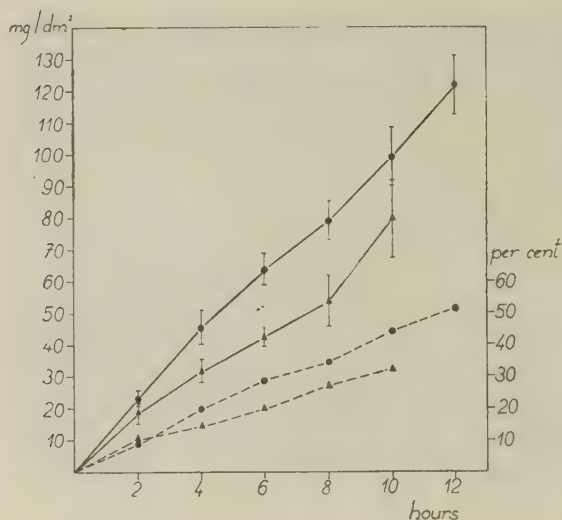


Fig. 6. Dry weight increment, in the course of 12 hours, of segments of the first leaf of two sorts of winter wheat. The vertical intercepts show the confidence interval ($\pm t 0.025 \times s_{\bar{x}}$). Full line — dry weight increment in mg./dm.². Broken line — dry weight increment in % dry weight (compared with the beginning of experiment). ● Weibulls Banco. ▲ Židlochovická osinatka.

of the light source from the sand surface being 50 cm.); the temperature during illumination was kept at $26 \pm 3^\circ \text{C}$, at night at $18 \pm 3^\circ \text{C}$. The plants were illuminated for 12 hours a day. The tenth day after sowing two leaves of equal size were removed from each pot early in the morning at the beginning of the light period. Segments were prepared immediately from the detached leaves, placed in a drying oven for 15 min. at 100°C and left there for further 10 hours at 60°C . The dry weight of segments was measured on a torsion balance with a scale divided into 0.01 mg. Table 1 shows the results in such arrangement giving always the weight of two control segments in juxtaposition to the weight of two experimental ones, which are otherwise placed on the support to be exposed to light for measuring photosynthesis.

It follows quite naturally from the statistical treatment of the experimental results that there is no significant difference between the two sets even at $P = 0.05$. It is of importance, however, that the difference between the K and E segments may be sometimes about 1% so that, in contrast to the original leaf-disk method, for a dry weight increment of 10% the accuracy of the result is $\pm 10\%$. The required accuracy of the original method (5%) may be achieved by increasing the number of segments. In spite of this, however, in some cases the application of two segments is satisfactory, as shown by the results of the experiment carried out with the aim of measuring the dry weight increment in the course of a 12-hour exposure (Fig. 6). Two sorts of winter wheat were used here: Weibulls Banco and Židlochovická osinatka. Cultivation of plants and removal of samples was identical with the data given for the estimation of the difference in dry weight between the control and the experimental set. Measurement was carried out on the 8th day after sowing. For each estimation 10 parallel samples of 2 segments each, i.e. a total of 20 plants, were used. The support with segments was placed in an exposure chamber constructed according to the authors of the leaf-disk method. The chamber temperature was 25°C , illumination intensity

about 70000 Lx, 1% carbon dioxide was added to conditioned air passing through the chamber.

It follows from the results shown in Fig. 6 that in the course of 12 hours the dry weight increment of the segments removed from the first leaves of winter wheat was linear under the given experimental conditions. Toward the end of the 12-hour exposure period the dry weight increment amounts to 51.5% in the sort Weibulls Banco, which corresponds to 122.5 mg./dm.². It is thus possible to apply this modification of the method to cereals for studying all the problems mentioned by the authors of the disk method.

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Использование метода дисков для определения фотосинтеза у злаков

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В работе предлагается видоизмененный метод дисков (Bartoš, Kubín, Šetlík 1960) для определения интенсивности фотосинтеза у злаков. Вместо дисков используются сегменты длиной 2 см. Из каждого листа при помощи специального приборчика чеканят сразу два сегмента, один из них служит для определения сухого вещества в начале опыта, второй экспонируется в камере при постоянных условиях. В связи с гетерогенностью листа необходимо для каждого повторения использовать хотя бы два сегмента из противоположных частей листа. Описана подкладка пригодная в предлагаемом видоизменении для кладки сегментов, чтобы обеспечить их насыщение водой. Приводятся приросты сухого вещества сегментов первых листьев двух сортов озимой пшеницы выращиваемой в песковых культурах при искусственном свете. Прирост сухого вещества в течение 12 часов линеарен и в случае сорта Вейбэлльс Бэнко (Weibulls Banco) достигает даже 51,5%, что отвечает приросту 122,5 мг-дм².

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J. SAHULKA: Free Amino Acids and Amides in the Axial Parts of Apple—Trees and their Relationship to Flower—Bud Initiation
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